ماجستير تناسليه (1)

Semen Analysis

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Advanced Semen analysis

Optional Tests

- CASA.
- Culture studies.
- Chemical "
- Chromatin "

SCSA
Sperm chromatin structure assay
Sperm Chromatin
Sperm assay

Sperm function Tests:

1. Sperm mucous interaction
2. " - Capicitation.
3. Zona Binding & Acrosome reaction.
4. Sperm ovum interaction. (SPA)

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1. CASA

(Computerized Assesed Semen Analysis)
(Computer Aided " ")

def. Semiautomated Technique composed of Video Camera & Computer to visualize & Analyze Sperm Concentration & motility (Velocity & Kinematics).

Adv. ① Percision is high.
② Provision of Quantitative data on sperm Kinematics.
② useful to detect effects of Toxicants on Sperm Kinematics & Morphometry (in Toxicology)

disadv. ① Expensive
② not accurate when Sperm Concentration is very Low or very high or when there are cellular debris.
③ hasn't shown to improve patient outcomes but rather, is helpful for research purposes.

Parameters or data delivered by CASA are:

① Curvilinear Velocity (VCL): Velocity along it bet. 2 succes.

(2) Straight line Velocity (VSL): Velocity along its straight path bet. 1st & Last positions (Measure For Forward progression)

(3) Linearity: $\frac{VSL}{VCL}$

(4) amplitude of Lat. Head displacement (LHD):

(5) Average path Velocity (.....)

(6) Flagellar beat frequency

(7) Evidence of Hyperactivation Motility ↑LHD ↓VSL

large amplitude of Head & tail + ↓ progressive motility → Measurement of Hyperachval

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· NB on LHD:

. dy: Width of the path taken by head during movement.

. Significance: Critical width of path taken by sperm head needed For penetration of Cervical mucous & oocyte envelope.

. the progressive motility is divided Acc. to the degree of LHD into 4 types:

- progressive motility with
  - minimal LHD
  - some LHD
  - Marked LHD
  - darting or whiplash LHD

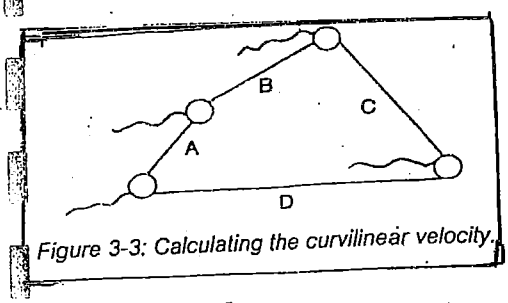


Figure 3-3: Calculating the curvilinear velocity.

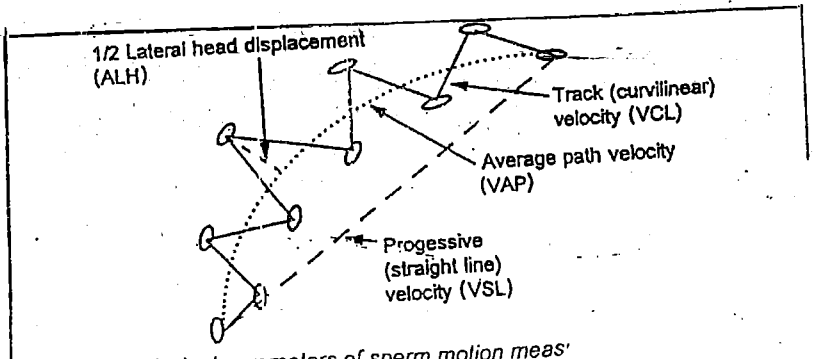


Figure 3-2: Principal parameters of sperm motion meas'

So CASA is valuable research tool useful in clinical work than!

2. Culture studies

may be needed for:

Semen

if there is evidence of infection or inflammation.

e.g Round cells (WBCs) > 1 million/ml or $\geq$ 10 / HPF

Urine

if there is evidence of urethritis or cystitis, prostatitis.

3. Chemical studies

(Optional tests to study)

Chemical markers:

- Epididymal markers.. (3) ?? مؤشر → α glucosidase
- SV markers: (2) مؤشر → Fructose
- prostate Markers: (4) مؤشر → Citric acid.

Sperm functions

By estimation of "ROS" level

- Origin:
- Level:
- Examples
  - NO^-
  - H_2O_2
  - Hydro
  - Hydrol

ROS (reactive oxygen species) & Infertility

- **Def. of ROS:** highly reactive oxidizing agents belonging to class of free radicals. a free radical is "any atom or Molecule that Possis one or More up paired electron"

- **Types of free radicals:**
  - H_2O_2 : Hydrogen peroxide
  - $\cdot OH$: Hydroxyl Radical
  - O_2^- : Super-oxide anion

  ROS ← Reactive Nitrogen Species (RNS)

- **Sources of ROS**
  - WBCs (main source)
  - Sperms (NL & abNL)

Effects (Functions) of ROS

Beneficial Effects:

Small amount of ROS can regulate

Harmful Effects

High levels of ROS → Oxidative Stress → may → Sper

Natural Defence Mechanism against ROS
(ROS Scavengers or antioxidants)

- **Enzymatic antioxidants:** (مضادات الأكسدة الإنزيمية)
  - Catalase
  - Superoxide dismutase (SOD)
  - Glutathion peroxidase. (GP)
- **Non Enzymatic anti-oxidants**
  - Vit E
  - Vit C
  - Uric acid.

the Scavengers are present in Semen in specific level. if the balance bet oxidant (Ros) & anti oxidant (Scavengers) disturbed ⟶ oxidative stress.

- **ROS & infertility:**
  - in fertile Men ⟶ ver low amount of ROS
  - in infertile Men (25%): ⟶ High level of ROS usually caused by leukocytospermia.

- **Lab assessment of ROS:**

4. Chromatin studies

(NL sp) (Sperm DNA Damage)

NL DNA of Sperm Means:

- No DNA fragmentation, oxidation (Nor) denaturation.
- Chromatin is: Stable or Condensed (NL Packaging)
- Histone replaced by Protamine (NB < NL Cells: Histones / Sperm: Protamine)

So Sperm DNA damage Means: DNA Fragmentation / AbNL Chromatin Packaging &/or Protamine deficiency.

→ Failed union bet ♂ & ♀ Gametes → No fertilization

[Sperms of infertile men have DNA damage > Fertile]

Causes of DNA damage:

Intra testicular (Dry testicular) Causes.

- Gonadotoxins
- Aging
- ↑↑ ROS e.g. infection

Extratesticular (External) Causes

- Chemotherapy
- Radiotherapy
- Smoking
- Varicocele

Indications For testing For Sperm DNA damage

1. Unexplained infertility
2. Recurrent pregnancy loss
3. Planning to have IVF

→ So Do "ICSI"

Tests For DNA Damage

↓

Sperm Chromatin Structure assay (SCSA)

↓ uses

- ↓ **Nuclear Chromatin decondensation Test**
  - ↓
  - decondensation by some substances as:
    1. Na dodecyl sulfate. → lysis of CM
    2. EDTA: → Chelate Zinc → decondensation
  - Decondensation ability differs bet fertile & infertile.
- ↓ **Aniline Blue staining**
- ↓ **Acridine orange staining**

↑ ↑

- The NL Sperm & NL chromatin will not take the dye (while) the AbNL Sperm with disturbed chromatin will take the dye.

Sperm Function Tests

. Introduction:

. Standard (Conventional) semen analysis is not an "accurate" diagnosis or prognosis of Human fertility in vivo or in vitro Except if { Azoo. 100% immotility 100% Necrozoo. } ∵ are uncommon causes of infertility.

. Most men ē infertility have ↓ Count, ↓ motility or abNL morphology; alone or in Combination (OAT); However; 30% of men have NL x Standard Semen analysis but They are classified as having "Unexplained Infertility"

def . so Sperm function tests aiming at: assessing The ability of sperm to:

1. Penetrate Cx mucus.
2. Traversing ♀ genital tract
3. penetration & Fertilization of oocyte.

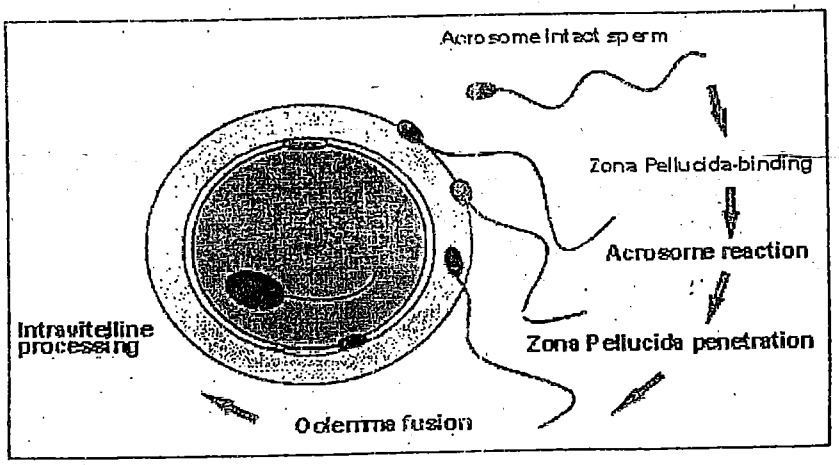


Figure 3-5: Diagrammatic illustration of stages of human fertilization. Spermatozoa swim through the surrounding medium and cumulus mass (not shown) and bind to the surface of the ZP. The acrosome reaction is stimulated by zona proteins and the acrosome reacted sperm penetrates the zona, enters the perivitelline space and binds to the oolemma via the equatorial segment. Oocyte processes surround the sperm head and it enters the ooplasm and decondenses. Infertility could result from defects of any of these processes, e.g. abnormal sperm particularly with defective head morphology bind poorly to the zona.

[1] Sperm-Mucous Interactions (اختبارات)

- **In Vivo** (Post Coital test) [PCT]
  - If -ve or abNL
    - Repeat
      - If -ve or AbNL
        - In vitro tests (Specially Cross over test).
- **In vitro** (SCMC)
  - Slide Test (Miller test)
  - Capillary Tub Test (Kremer test)
    - If -ve
      - do.
        - "Cross over test"

InVivo Sperm Mucous interaction Test (Post Coital test)

- **Aim** to evaluate Sperm ability of <Penetration & Viability> inside the ovulatory endocervical Mucous in (9-24 hrs) after Coitus.

Precautions: ① done Exactly at time ("day") of ovulation (detected by):

- For ♂ → 4 ds
- For ♀ → ovulation time
- For Both → Abstinence

- BBT
- Serum Estrogen
- Vaginal UIS
- Modified Insler score of Cervical Mucous:

② done within 9-24 hrs after Coitus (2-8 hrs) التحريض

③ 2 Samples are obtained (by) Non Lubricated Speculum from <Vaginal Pool / Endocervex>

④ Abstinence of 4 ds.

NB Insler Score of Cervical Mucous

- Mic exam of ~~Cervical~~ Mucous; then assessment of:

1. Volume
2. Viscosity (Consistency)
3. Ferning (Crystalization) 
4. Spinn barKeit (Elasticity)
5. Cellularity.

↓

Score given for each of these 5 parameters

(Ferning ←) شكل الكرستالات بتاعة لما يتوضع على شريحة

(Spinn barKeit ←) شكل "Elasticity" when stretched bet. 2 glass slides.

- **Volume score:**
  - 1 : 0.1 ml
  - 2 : 0.2 "
  - 3 : ≥0.3 "

- **Viscosity score:**
  - 0 = Highly Viscous (as in Premenestrual Mucous)
  - 1 = Intermediate
  - 2 = mild
  - 3 = minimal or Watery (Preovulatory Mucous).

- **Ferning (Crystalization):**
  - 0 = No crystalizat^
  - 1 = Atypical "
  - 2 = 1ry & 2ry Stem ferning
  - 3 = 3ry & 4ry " "

- **Spinn barKelt (Elasticity):**
  - 0 = length of stretched mucous < 1cm
  - 1 = 1 – 4 Cm
  - 2 = 5 - 8 "
  - 3 = ≥ 9 cm.

: Cellularity:

0 = > 20 WBCs - other cells / HPF
1 = 11 - 20 Cells / HPF
2 = 1 - 10 Cells / HPF
3 = 0 Cells.

Score
- Maximum = 15 ✓
- if < 10 unfavorable mucus
- if > 10 good Mucus.

Interpretation & Significance

- Vaginal Pool Sample:
  - +ve Sperms → successful semen deposition by ♂
  - -ve " → Failed deposition (Coital & Iter Mechanical Infertility)
    - d.t < ♂: Deposit= disorders (ED, PE)
    - < ♀: Mechanichal disorders (.....)
- Endocervical Sample:
  - +ve or NL test → 10-20 >5-15 progressively motile Sperms / HPF.
  - Poor or abNL Test → < 5 Sperms / HPF (specially ē Circular or Sluggish movem).

↑ Viscosity or delayed Liq. ← ♂ = ♂+♀ Idx → ♀: AbNL Cx Mucus.

Causes of poor postcoital test:

a. **Male factors:**
- Improper semen deposition e.g. erectile or ejaculatory dysfunction.
- Poor semen quality e.g. poor motility or count, increased viscosity, or delayed liquefaction.

b. **Female factors:**
- Mechanical causes: Vaginismus, vaginal septum or abnormal cervix e.g. pin-pointed os.
- Abnormal cervical mucus.

c. **Sperm antibodies (immunologic interactions).**

To differentiate between male and female factors in-vitro, Sperm-Cervical Mucus Crossed Invasion test is done.

Euromed

NB: inappropriate Timing (IS) the Most Common Cause of AbNL PCT
- its Value → Controversy (ARTs ...)

In vitro tests (اختبارات)

(Sperm Cervical Mucous Contact test) (SCMCT)

Slide test (Miller kst)

drop of husband semen (ejac. in 2 hrs)
+
drop of wife's cervical mucous (at time of ovulat.)
↓
put in close (contact) on slide & covered by cover slip
↓
Results

1. NL or good → rapid invasion by large No of Actively motile Sperms in 15 min

2. Poor: Few or No penetration ± d.t either:
   - Abnl Semen (↓ motility)
   - Abnl Mucous (↑ Viscosity)
   - "so" → to differentiate "Cross over Test"

3. Abnl test: penetration but either immotile or shaking يتزلزل

↓
Immunological infertility

Capillary tube (Kremer) test

. See the apparatus.

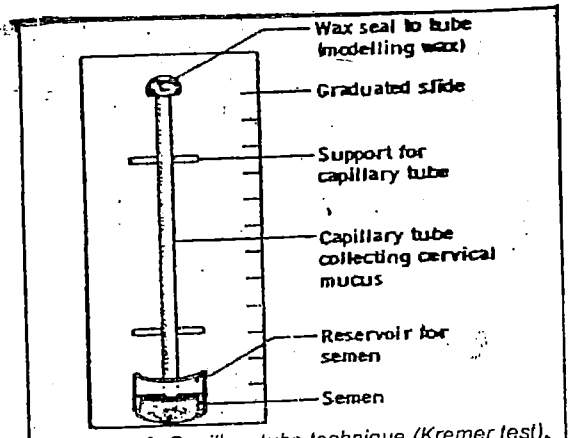


Figure 3-6: Capillary tube technique (Kremer test).

. tube is incubated in a moist enviroment For 1 hr.

Results the test will be +ve if:

1. No of penetrating Sperms ≥ 5o /HPF
2. distance of penetration ≥ 5o mm/hr.
3. duration of progressive motility should be at least for 24 hrs.

. has Better Prognostic Value for in Vivo fertil.
. Better > post coital.

Euromed

Cross oVer Testing [Cross Mucous Hostility Testing]

Aim: if PCT is -Ve or AbNL The Etiology ± abNL Semen or AbNL Cx Mucous to differentiate

Partners Semen tested

- Cervical Mucous of NL fertile Donor
- Partners Cervical Mucous.

&

The Partners Cervical Mucous tested

(It replaced by bovine Mucous)

- Semen of NL Fertile donor
- Partners Semen

NB Indications of PCT:

- unexplained infertility
- High Viscosity
- delayed Liquefaction
- High Vol or low Vol @ NL Count

II. Tests for Assessment of Fertilizing Capacity:

1. HOS Test (& ROS)
2. Sperm Capicitation
3. Zona binding
4. Acrosome Reaction
5. Sperm Penetration

[1] HOS test → see before

[2] Sperm Capicitation:

- Sperm Washing → incubation in Culture media containing Human Albumin → CASA Assessment

Hyperactivation Pattern of Motility (Vigrous type of Motility) means:

1. High Curvilinear motility
2. low linearity
3. High amplitude of LHD & Tail displacement

- Results of this:
  Test → Correlated ē preg. test in ART

[3] Zona Binding assesment (HemiZone assay):

- Binding of sperms to ZP (Zona binding surrounding ova) → Acrosome Reaction.
- For assesment of this Binding: "HemiZone Test"

Non living, Non Fertilized Human oocyte is divided into 2 halves:

- ✓ 1 half + Capicitated Pt. Sperms
- ✓ 2nd half + capicitated NL Fertile donor sperms.

No of pts bound sperms / Donor bound sperms X 100 = Hemizone Index (if <30%-40% → ICSI (not) IUI)

NB 1. THP test : is an excellent predictor for fertilization rate in ART

2. Cloning of Recombinant Glycoproteins of Zona (ZP3 & ZP2) Recently used will facilitate wide spread of test.

4] Acrosome Reaction assessment:-

- detection : 1. E/M (Expensive & Labor intensive)
  2. Immunofluorscent staining + HOS Test (ICSI)
  3. Monoclonal Anti-bodies.
  4. Fructose Containing Ags (Recently used as a markers for AR).
- x. disadv 1. very expensive
  2. Labor intensive
  3. Subjective ذاتية
  4. non cost effective
  5. represent only 5% of Infertility Causes. (Failed reaction)

- The induction can be done by: "Ca+ Inophore" ;

- Assessm of
  - Spont (in)
    - pts
    - NL
  - Induced

- In Fertile Men
  - (SAR) Spont. Reaction rate < 5%
  - (IAR) Induced Reaction Rate 15-40%

- in infertile Men
  - Spont. Reaction Rate is Higher
  - Induced Reaction Rate is Lower.

(Wpl AR type)

- Indications of this test:
  detection of Acrosomal defect (in) pts. ē unexplained poor IVF results so do → ICSI

- NB : Acrosomal Index % =

(Size, Form, Storing Capacity)

$$\frac{\text{Spermatozoa ē NL Acrosomes}}{\text{Total No of Spermatozoa}} \times 100$$

Sperm Penetration Assay (SPA)

(Zona Free Hamster egg penetration test)

Introduction:

- last step of Sperm-ovum interaction
- involves binding of sperms to oocyte mem.
- → Fusion bet. 2 membs. → Penetration of sperm Nucleus into oocyte Cytoplasm.

↓

To do this test:

Hamester eggs (Zona free) (after removal of < C. oophorus / Zp as Zp prevent cross species fertilization)

+

Sperms (of Pt.) (after washing, capicitation induct° & suspension in BWW Medium)

↓

- mixing in culture media
- Incubation for 3 hrs at < 37 °C & 5% Co2
- oocytes: washed → Fixed → stained & Examined Mic. for Sperm penetration.

- This test requires the sperm to be able to Undergo
  (1) Capicitat°
  (2) AR
  (3) Fusion dolemma
  (4) interoperat° to ooplasm.

Interpretation:

- NL test → 10-30% of ovae are penetrated (so can fertilize Human ova)
- Abn'l → < 10% of ovae are penetrated (Infertility) → So IUI or IVI not suitable
- Zero test → Major Impairment of Sperm function Capacity. → DO ICSI

Exclusion Criteria for IVF or IUI

- disadv: (1) Not frontline clinical diagnostic Test & its value is unquestioned.
  (2) don't evaluate Zp penetration.

- Indicat°: (1) unexplained infertility to decide IUI/IVF or ICSI
  (2) Pt Candidate for regular IUI or IVF but have low Morphology Sperm.

Factors affecting the out Come of SPA:

1. No of motile sperms
2. Pattern of Motility: chance of penetration
   ↑↑ with < progressive straight speed (>25 μm/s)
   small amplitude of LHD
   [However NL penetration can occur e⁻ = Immotile Cilia Synd]
3. Ability to penetrate Cervical Mucus.
4. Ability to undergo: < Capacitation
   AR
   Fusion e⁻
   Vitelline memb.

Semen Components

During ejaculation, these components are not discharged simultaneously, but in a predetermined sequence. Thus, by carefully collecting semen as it is discharged, it is possible to separate out the secretions that make up an ejaculate "split ejaculate" to make use of the 1st portion in techniques as AIH.

At first, secretions from bulbourethral (Cowper's) and urethral (Littre's) glands, rich in mucoproteins, come forming the **prosemen** essential to neutralize urethral acidity before ejaculation.

Semen components	
1. Sperms and secretions from the testes and epididymis	5%
2. Seminal plasma:	
a) Prostatic secretions	30% (10-30) (0.5 ml)
b) Seminal vesicles secretions	60% (40-80%)
c) Cowper's glands and Littre's glands	5% (2-5%)

Split ejaculate

	1st portion	2nd portion
Source	Prostate, testes & epididymis	Seminal vesicles
Coagulation	Absent	Present
Liquefaction	Rapid	Delayed (20-30 min)
Viscosity	Less	Higher
PH	Lower (acidic)	Higher (Alk)
Sperms	High conc. & of better motility	Low conc. & less motility
Biochemical products	(A) Prostate: Acid phosphatase, citric acid, zinc, proteases, spermine	Seminal vesicles: Fructose, prostaglandins, protein-like material (for semen coagulation)
	(B) Epididymis: Carnitine, Inositol, Sialic acid, Glycerophosphoryl choline, forward motility protein.	S.V. secretions may contain suppressive factors → deleterious effects on the sperms.

Clinical uses of split ejaculate

1. The 1st portion is used for AIH in cases of high viscosity, large volume, oligozoospermia, asthenozoospermia.
2. Tracing the origins of various compounds in seminal plasma e.g. fructose, PG ... etc.

Prosemen (Cowper, Littre) (5%) → then → First fract.: Testis, Epid., Prost. 5% + 30 → then → 2nd fract.: SV (60%)

Semen Analysis

Basic Introduction:

- Semen Analysis **Is not** a test for Fertility but is a predictive indicator for **functional** status of ♂
  - reproductive Hormonal Cycle
  - spermatogenesis.
  - patency of genital tract.
- **The only True & Sure test** for Fertility is Initiation of pregnancy.

- **Keep in Your mind**: that NL values have been difficult To be determined for Fertile men during their reproductive period **but** clinical studies of Infertile **males** have established "**Limits of Adequacy**"

(NL Semen) إلى حد بعيد عن معلومات بسائل المنوي الطبيعي . إلى حد ما أقل نطاق بحيث يحمل حيوية وليس مستحيل"

(So subfertile Not infertile Except if Azoo. → infertile or Sterile).

- **So these limits are not absolute why??**

1. Some fertile male have semen quality **below** these limits & some Infertile males having semen quality within NL Limits of Adequacy.

2. Standard Semen analysis doesn't asses the Functional **integrity** of sperm (Standard analysis may be NL **but** sperm function tests may be AbNL → Failure of Conception)

"سفي" NB: 30% of men ē Adequate Standard Semen analysis → Having abNL Sperm function. (Hence they are: infertile)

Semen Evaluation
(Semen Analysis)

- **Standard Semen Analysis** (Conventional)
  - ١. كفاءة تجميع العينة
  - ٢. Physical Exam
  - ٣. MiC Exam
- **Advanced Semen Analysis**
  - Optional Tests
  - Sperm Function Tests

18 Points .. هما نقاط ب

Standard Semen Analysis
(Limits of Adequacy)

A. Sample Collection

1. Abstinence period: (الامتناع)

- 2-7 days (Typically 3-4 ds)
- if < 2 → oligozoo. &oligospermia
- if > 7 → polyasthenozoo.
- Abstinence is the major source of variation in semen parameters [sperm count ↑↑ 25% / day]

2. Perform 2 Analyses:

- لازم المريض يكون عمله ٢ تحليل سيمن مختلفين
(2 analyses 1-3 ws apart) → (2-4 ws) اذا آخر

3NB

- Why doing 2 analyses? because men have great Variations in Their Semen Parameters.
- If there is great Variation bet. the 2 → (do 3rd Semen Analysis)
- "يعنى" Q. ماهى الحالات اللى ممكن نعيد فيها التحليل بعد فترة ٣ شهور ؟؟

↓ if.

1. Exposure to high fever or illness e.g. الانفلونزا
2. Exposure to Cytotoxic agent or drugs.

(Method)
- Masturbation or Collecting Condom, Foreplay at Home
- Coitus interr. or ordinary Condoms

(site) < lab or Home.

3. Collection Method:

- Should be at Lab (but) if not at home Collect (by Coitus interruptus) (but Transport it ē in 1-2 hrs) — d.t anxiety of < Masturbation ! lab.
- obtain it by: Masturbation (Should be dry) but non spermicidal Vaginal lubricants may be used (as Vasiline or Olive Oil)
  - (dry) ← ممنوع استعمال: الماء، الصابون، اللعاب، K-Y gel
- Avoid
  - Condom: Contain spermicidal substance
  - Coitus interruptus ??
    - prasemen may Contains SpermatoZoa ??
    - 1st fraction of ejaculate Contains very much SpermatoZoa
    - Contaminate by Vaginal acidity & secretion.

NB
- Special Condoms Now used called "Collection Condoms": made of Silicon or Poly urethan (as Latex is spermicidal).

4 Container & Temp

بعدما اخرج ؟؟ → 4

- Clean (plastic or glass) not Toxic to SpermatoZoa.
- 20 - 40 °C (room - body Te)
  - انتقل حاملا العينة في جيبه قريبة من جسمه

5 Period before Exam: (لازم يكون ساعه بعد جمع العينة
وكذلك ينقل سريعاً للمعمل)

- If the previous semen was NL, Period —fix→ within 1 hr.
- If the previous semen was abNL —"→ < 1 hr
- If Microbiology is Needed —"→ ē in 3 hrs.

⑥ Culture: If Culture is Needed; the patient should Pass urine then wash his hands & penis before ejaculation & microbiology should be performed ē in (3 hrs)

NB: split ejaculate collection may be Needed.

B. physical Examination

1. Color
2. odour
3. Volume
4. Liquefaction
5. Viscosity
6. PH.

1. Color:

- NL → grayish opalescent
- AbNL:
  - whitish: → Excess No. of sperm or WBCs.
  - greenish → Genital inf.
  - Yellowish →
    1. Long abstinence
    2. Urine Contamination → BN drug.
    3. Bilirubin → LCF
    4. Yellow + clumbs + bad odour → inf.
  - Red →
    1. Drugs → Rifampacin
    2. Blood (Hemospermia):
       - pink -------→ Fresh Traces
       - Bright red → Fresh Excess
       - Brown → old Excess.

2. odour: Chic odour is d.t Spermine of Prostate.

3. Volume:

- NL: 2 – 6 ml (≥ 1.5 ml)
- Abnormalities may be:

spermia = seminal fluid

Zoospermia = sperm

*** Aspermia**
(Absent seminal fluid)
||
Anejaculation (فيما بعد)

*** Low Volume** (< 2 ml)
- Oligospermia
- Hypospermia
- Parvisemia

Causes:
1. short abstinence period
2. Faulty collection
3. Accessory gland dysf. or Atrophy. (Chr. TB or Bil. → fibrosis)
4. RGE, retrograde
5. ejac. duct obst. (unilat.)
6. Androgen deficiency → Accessory glands Atrophy.
- Seminal vesicle disorders

*** Excess Vol** (> 6 ml)
(Hyperspermia)
Polyspermia
- Causes:
  - long Abstinence period.
  - Contamination by Urine
  - Acute inf.
- Effects:
  1. relative Oligozoo.
  2. loss of Large port of semen after intercourse.

4. Liquefaction:

Semen after ejaculation has 3 Forms:

Liquid —Immediately→ Coagulated —"في دقايق" 20–30 mins (< 30 mins)→ Liquefied.

Coagulated:
- مسئولة: SV: dit. Their proteins (as Kinase or Semenogelein)
- Failed in: SV disorders
  - CBAV
  - ej. duct. obst. (EDO)

Liquefied:
- مسئول: Prostate d.t their Liquefying enzs.: (Proteases = Fibrinolysins)
  - PSA
  - PAP
  - PBP
  - Tissue plasminogen Activator (From Cowpers)
- Failed or delayed Liquefaction (> 30 mins) → prostatic disorder e.g. Prostatitis.
- appear as: "Heterogenous gelatinous clots inside Liquid"

> Semen should be liquefied ē in 20–30 min
> if > 30 → delayed Liquef.

- Liquefaction & infertility
  - To diagnose delayed Liquefaction as a cause of infertility. Sperms should be absent in post coital test.

- Treatment of delayed Liquef. (>30 min) → AIH

After Exclusion of Prostatic disorder → Add Liquefying enzs (0.2% Amylase, α-chemotrypsin, Hyaluronidase) → Then → A.I.H

5. Viscosity

- def: resistance of the fluid (semen) to flow or its "Thickness"
- Estimation: By allowing the completely Liquefied (30 min) Semen To be poured Through wide bored 5mm pepitte

NL Viscosity (Normoviscous)

- Semen Comes as discrete drops (drops by drop) from The Pepitte or < 2Cm thread.
- Grading: From:
  - 0: consistency of water (To)
  - 4+: " " gel.

Note that

High viscosity

- Semen Comes in Threads > 2 Cm from The Pepitti.
  or
- quantified by measuring The time needed for one drop to leave The Pepitte. =
  - Etiology: SV disorders
  - Evidence ↓ Fructose and ↓ Hyperviscous (Semen)

. Viscosity & infertility:

. role of ↑↑ viscosity in infertility is controversy as many patients with viscous semen can achieve conception.

So . don't consider viscosity as a cause of infertility

Except if there is:

1. Persistent high viscosity (داعاة يتكبير لاتزق)
2. Absent or very low No of sperms in postcoital test (PCT)

. High viscosity may lead to (effect on semen):

(Margin note: repl. → ↓ motility; ↑ chromatin stability)

1. Easy drainage after intercourse (تصريف جوه cervix د)
2. Failure of coating the cervix
3. ↓ motility → inability to enter cervical canal.
4. ass. ⓔ High chromatin stability.

N.B. to differentiate bet. high viscosity & delayed or failed liquefaction:

- Failed liquefaction → Heterogenous gel masses in more fluid base.
- High viscosity → ↑ thickness & come in thread > 2 cm from the 5 mm bored pipette

. Treatment of high viscous semen: → AIH

- . Addition of enzymes (as in liquef.)
- . " of culture media. (... الكثيفة)
- . Repeated needling: repeated passing of semen through "19 G syringe" (avoid it as it → deterioration of semen motility).

then → (AIH)

If you see < delayed Liquefaction: / or / High Viscosity. → Repeat Semen analysis & Exclude < prostah / SV causes

If persistant → Post Coital test ← No Viscosity ← sperm Go عنق cervical mucosa or not acquire of infertility

6. PH of Semen:

- NL: 7.2 - 8 (Alkaline) [d.t bicarbonate] → antagonize Vaginal Acidity. seminal vs. secretion

- Note that:
  - Prostatic sec. → slight acidic (6.5)
  - SV sec. → Alkaline.

⇒ So: Alkalinity of Semen is d.t SV Secretion.

- Abnl PH:
  - < 7 + Azoo. → CBAV · SV obstruction Hypofunction
  - > 8 → Genital infection.

NB: Abnormalities of SV & prostate: may alter the PH.

SV يفرز: Volume, Coagulat^, Viscosity, PH → So in CBAV or ejact. duct obst:

- ↓ Vol (Hypospermia)
- Failed Coagulat^
- ↑ Viscosity
- ↓ PH (Acidity)
- Azoospermia.

if you see < delayed Liquefaction / or / High Viscosity. → Repeat Semen analysis & Exclude < prostah / SV causes

If persistant → Post Coital test ← No Viscosity ← sperm or not acquise of infertility

Go cervical mucosa

6. PH of Semen:

- NL: 7.2 - 8.8 (Alkaline) [d.t bicarbonate] → antagonize Vaginal Acidity.

seminal ves. secretion

- Note that:
  - Prostatic sec. → slight acidic (6.5)
  - SV sec. → Alkaline.

لازم تسيل بعد liquefact

- So: Alkalinity of Semen is d.t SV Secretion.

- Abnl PH:
  - < 7 + Azoo. → CBAV
  - > 8 → Genital infection.

- SV obstruction
- " Hypofunction

NB: Abnormalities of SV & prostate: may alter the pH.

SV تفرز: Volume, Coagulat^, Viscosity, PH → So in CBAV or ejact. duct Obst:

- ↓ Vol (Hypospermia)
- Failed Coagulat^
- ↑ Viscosity
- ↓ pH (Acidity)
- Azoospermia.

"Microscopic Examination of Semen"

(6)

1. Concentration (Count)
2. Motility
3. Viability
4. Morphology
5. Agglutination
6. Non Sperm Cells

---

1. Sperm Concentration:

لازم تواجد هاتين القيمتين معاً

- Sperm Conc: 1ml : should be > 20 million/ml (20 - 250 200)
- Sperm Conc/ejaculate (Total Count/ejaculate) : should be > 50 million.

هاتين القيمتين لهما نفس الأهمية بدليل :

. If Sperm Count/ml = 15 million/ml $\xrightarrow{So}$ "AbNL"

& if ejaculate vol. is 5 ml, So total count/ejaculate will be 5 ml × 15 mill. = 75 milion $\xrightarrow{So}$ "NL"

. So the 2 values should be at NL to fulfill the NL semen analysis.

. Abnormalities in Count may be:

1. AZooSpermia → Complete absence of spermatozoa
   - Testis
   - transport
   - No Azo

"قاعدة" AZooSpermia Not Considered Except after Repeated Centrifugation of Semen

2. Hidden (مخفي) CryptoZooSpermia: → If the semen sample is AZooSpermic → Repeated Centrifugation, If there are sperms in the sediment → Called "CryptoZooSpermia"
   If No sperms detected → AZooSpermia.

. NB: (CryptoZoo = Hidden Spermatozoa = apparently Absent Spermatozoa)

3 **Oligo Zoospermia** (Count < 20 million / ml or < 50 mill. / ejaculate)

Causes

False

1. Short Abstinence period
2. psychogenic Incomplete ejaculation (anxiety)
3. Loss of 1st part of ejaculation (Faulty collection).
4. Hyperspermia: large vol. of seminal plasma → relative oligozoo.

True (persistent oligozoo.)

1. **Idiopathic**: Commonest cause.
2. **Genetic**:
   - AR inheritance usually present
   - usually ass. ē severe oligo (< 5m/1ml)
3. **Obstructive**: Partial or unilat. ejac. duct obst.
4. **Impairment of Spermatogenesis**
   - Varicocele.
   - Infection
   - Drugs.
   - fever
   - systemic illness.
   - smoking
   - orchidopexy
   - Antisperm antibodies
5. **Isolated FSH deficiency**.

NB

Severe oligo zoosp. (< 5 million/ml)

↓ do

(Specially if ICSI is indicated)

A. **Genetic Screening**:
- Karyotyping (if < 10 million)
- Yq micro deletions (if < 5)
- CFTR = cystic fibrosis Transmembrane Regulator.

B. **Genetic Counseling & Hormonal profile**

Treatment of Oligo Zoo

- Treatment of Treatable Causes.
- Non treatable causes → ICSI

NB: Hormonal tt now considered of No Value (Except if FSH deficiency) as
- Gonadotropin inj.
- Clomiphene
- Tamoxifen
- Testosterone.

4 Poly Zoospermia: (Count > (200) or (250) million / ml)

- AET [False: physiological polyasthenozoosp. d.t Long abstinence period.
  True: persistent.

- Role in infertility: Controversy but ± ass. with:

↓ Fructose (Consumption by large No.) → ↓ motility

(1) repeated analysis. (to exclude false..)
(2) Repeated ejaculation in vitro stim. of motility & AIH.

(2) Spontaneous Abortion in wives
3. Variable results of Hamster egg penetration test.

NB: Methods of Sperm Count

(1) Rough method
(2) Hemocytometer
(3) Makler Counting Chamber
(4) Electronic Counter
(5) DNA Flow Cytometry

1. Rough method: (No. / HPF X 10^6):

- Counting the mean No. of sperms in several fields under a (40x) objective & multiplying x 10^6

eg 60 sperm / HPF $\xrightarrow{\text{roughly it is}}$ 60 million / ml

- Should be done during intial Exam. to determine the dilution needed during hemocytometer Counting.

2. Hemocytometer:

- The most accurate method

- عبارة عن شريحة مقسمة الى مربعات كبيرة ($1mm^2$) كل مربع كبير مقسم الى ٢٥ مربع صغير يبدأ بختار ٥ من المربعات الصغيرة الموجودة في المربع الكبير اللي جنب الشريحة وتعد الحيوانات المنوية

- at first before Counting:

1. Spermatozoa should be immobilized

1:20 → إذا Sperm Count (by rough method) >50 /HPF

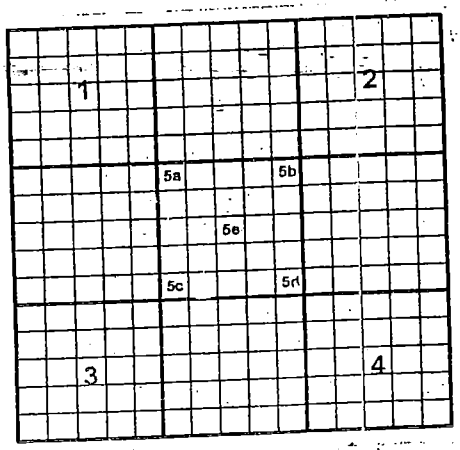
1:10 → إذا Sperm Count (N N H) is <10 /HPF

بنضرب لتحويل عدد الحيوانات الـ ٥ مربعات صغيرة X ٥

بجمع العدد في ٥ مربعات صغيرة ونقسم على ٢ X 10^6

NB

Only recognized Spermatozoa are counted not the Free Sperm tails



(Hemocytometer)

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3. MaKler Counting Chamber :-

- Special Counting Chamber for rapid Semen analysis have become popular & gained the widest recognition & use.
- No dilution is used So, No of Sperms Counted / 10 Squares = Sperm Conc. in millions / ml.

(Causes / Errors) → . Not recommended for routine use & when WHO Standards are desired but used as a simple method for less Specialized Labs.

4. Electronic Counter: Easy but expensive.

5. DNA Flowcyto

. rapid & precise
. haploid sperm differ in Staining ch than othe. diploid cells in Seme

2. Sperm motility

For assessment. شروط ٣ — Should be Assesed

1. at least 200 Spermatozoa Should be sCreened
2. should be immediately after ejaculation & 1 hr after that (preferably by 30 mins after ejac). (30–60)
3. should be both
   - Quantitative ✓ نسبة الحيوانات المتحركة.
   - Qualitative ✓ تحديد نشاط الحركة.

شروط

(EAU)
- 30–60 mins after ejac.
- 37°C

I. Quantitative Assesment

- Counting the motile & immotile in at least (5) fields
- $\% \text{ of motile} = \frac{\text{No of motile}}{\text{Total No of Sperms}} \times 100$
- (Impor) NLLy: at least 50% should be motile. (≥ 50%) show progressive motility.

II. Qualitative assesment

Subjective Methods

I. Acc. to degree of Forward Progression:

- 0 : None: non motile
- 1 : Poor: weak forward Progressive
- 2 : Mod: definite "
- 3 : Good: Good "
- 4 : Excellent: Vigrous "

II. Acc. To WHO 1999: ✓

- Grade a : Rapid progressive (≥ 25 μm/S)
- " b : Slow (sluggish) " (5–25 "/")
- " c : Non progressive (< 5 μm/S)
- " d : Immotile

"Flagellar activity."

Objective Methods → CASA (الحسابات)

1. Prolonged time Exposure photomicrography: photograph show Tracks produced by sperm head accross The field during 2 sec. exposure
2. Multiple Exposure photography: successive photographs of sperm along the field & from change the position; Velocity can be calculated.
3. Video micrography.
4. Laser light.
5. CASA.

- Normally: ≥ 50% Should be progressively Motile

≥50% progressive (good)

or

- Acc. To WHO { Ga ≥ 25% or Ga+b ≥ 50%

- Asthenospermia: if [Ga < 25% or Ga+b < 50%

.. Causes ??

See infertility

- inf.
- Varicocele

Motility if:

- NL → Means:
  - ≥ 50% progressively motile
  - Ga ≥ 25% or Ga+b ≥ 50% (WHO)
- AbNL (≥40% immotile)
  - Immotile — Severe Asth. <5% — Ex <40%
  - Viability test
    - Necrospermia → E/M
    - Astheno-spermia → do

Severe Asth. <5%

NB

- (a) = Progressive Motility → Strong swim fast in straight line
- (b) Non-linear " → move forward but in curved or crooked Motion

Motility disorders

→ >50 should p—

GA > 25

Gb → 10

Ga+b → 35

3. Sperm Viability (Vitality) ... → Asthenozoosp.

def. Immotile sperms may be < living or dead (→ Necrozoospermia) to differentiate bet. them do these tests..

Indications:

1. Very low No of sperms
2. if > 40% graded d sperms are immotile to differentiate bet immotile living (Asth.) & immotile dead (Necrozoosp).

(3) very low or absent Mot. < 10% (5-10%)

Idea: depends on the Cell-membrane status:

- Intact → in living sperms (so don't take the dye)
- damaged → in dead sperms (Take the dye).

Tests for Vitality:

1. Dye Exclusion method (N/E stain)
2. Hos test
3. Invitro stimulation. مهم

طريقة أولى

0.5g EY
3g N
30 ml distilled water
N/E sol
2 drops
Semen drop.

- 1 drop → Semen (100 spermatozoa) fix
- 2 drops → Eosin Y 1%
- 3 drops → Negrosine 10%

→ all mixed on glass slide Then a drop be put on another slide & Examined under phase contrast Mic.

Result <
- living sperms (intact memb): will not take the dye → appe White
- dead sperms (damaged memb): will take the dye & appear as red (d.t E) against the Violet background (d.t Neg).

(2) "Hos" test (Hypoosmotic Swelling): Hypoosmotic Solution (150 mmol/L) + Spermatozoa →

Result / Living sperms: (Semipermeable memb / maintain osmotic equilib) → (bulging) of plasma

يدخل الـ Fluid جوا membrane & curling of tail.

\ dead sperms: (leaking memb) → No swelling (retain its morphology)

يدخل Fluid و يخرج تاني due to damage of memb.

③ Invitro stimulation of Motility: by using Kallikrein or Caffeine.

NB. * These tests used during ICSI to select the Immotile But Living sperms.

* in cases of NecroZoo → Obtain sperms from testis (viable.)

IV * Apoptosis: an important mechanism of NL Spermatogenesis & its disturbance → impaired quality.

. Staining of Apoptotic sperms by:

- Staining
- Flow cytometry
- Terminal deoxy nucleotide mediated dUTP nick end labelling (TUNEL).

. % of Apoptotic sperms (0.1% - 50%)

Normal Viability:

Sperm vitality or viability should be ≥ 75% → (> 75% of sperms should be living)

لو اقل ← Necrozoospermia

4. Sperm Morphology

1. 2 Criteria (For assessm % of NL) → WHO / KSCM (Kruger Strict Criteria of Morphology)
2. 2 MiC Exam → LM / EIM
3. 3 Indices → TZI, MOI, SDI — TZI > Roy's Acro-Somal index.
4. 3 Anomalies → OAT / Globozoospe. / Sterelizing Sperm defect.

---

① 2 Criteria used for Assesment Morphology : (100 Spermatozoa evaluated). or (better 200)

الأكثر استخداماً

WHO

NLLY : NL Forms should be ≥ 30%
(NL Semen) & AbNL ~ should be < 70%

- So Teratozoo Spermia Means:
  NL Forms are < 30% → WHO
  < 14% → KSCM

KSCM

- depends on: Counting The Border line Sperms as abNL (WHO Border line → NL) So Called "Strict"
- if > 14% of Sperms are of NL morphology → NL Semen
- Reference of NL Sperms: sperms from endocx & Zona binding.

② 2 methods of Morphology assesment:

A. LM → after staining with:
- H & E
- Eosin
- Giemsa
- Papanicolaou (الأفضل والأهم) (تنظيري)
- Leishman stain
- Shorr.

WHO Recommend: . pap . Shorr . Diff Quick stain

B. EIM → to diagnose ultrastructure defects. e.g Axonemal defects.

Higher predictive Value for determining rates of pregnancy in IVF
if KSCM > 14% → 91% FR
< KSCM < 14% → 37% FR.

. See Criteria of NL & Varieties of AbNormal Sperms

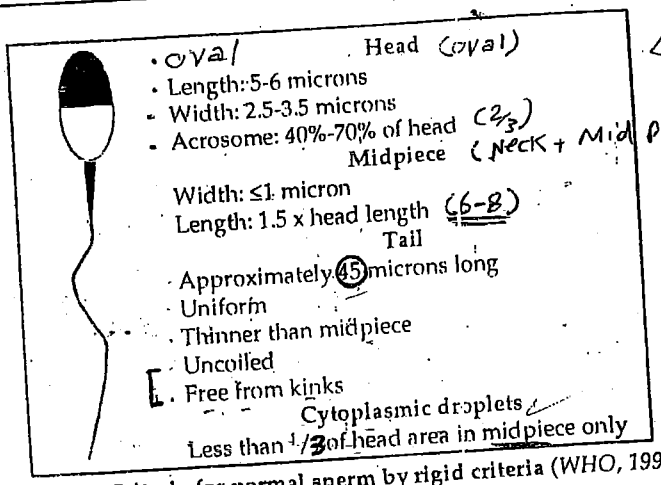


Fig. (24): Criteria for normal sperm by rigid criteria (WHO, 1999).

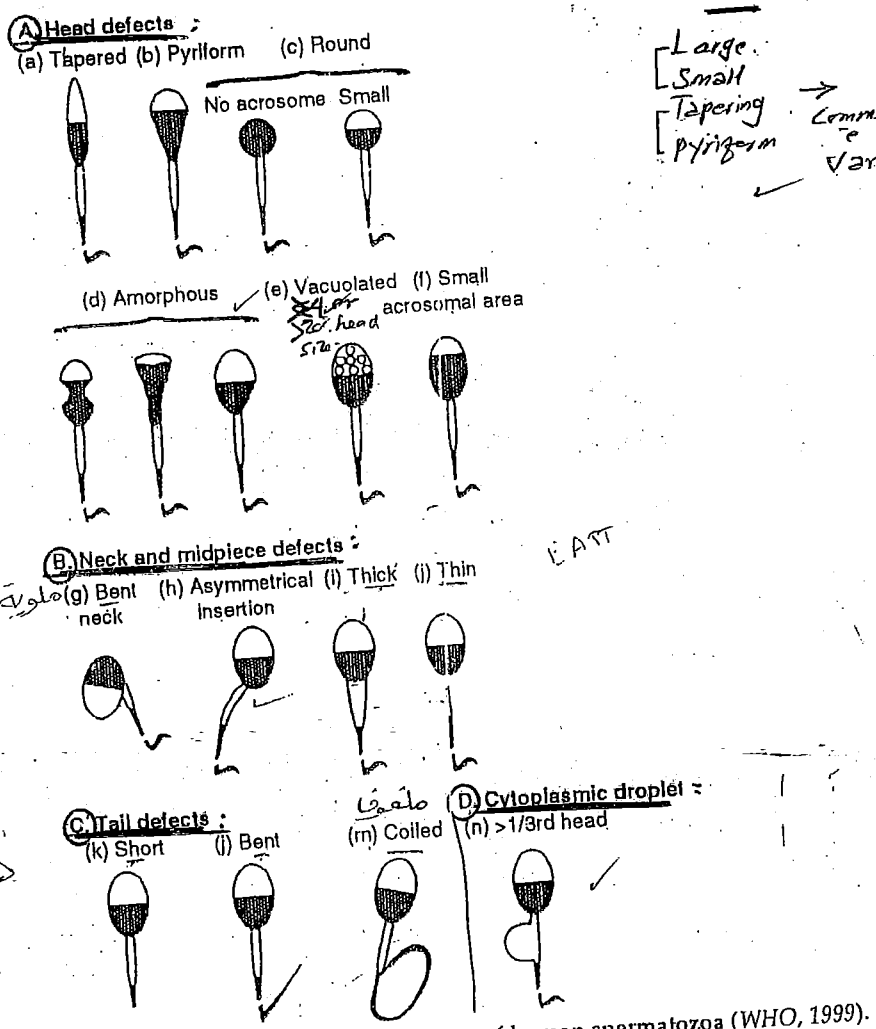


Fig. (25): Schematic drawings of some abnormal forms of human spermatozoa (WHO, 1999).

4 Sperm Agglutination & Aggregation.

- **Sperm Agglutination** (if > 10% → Significant): living, motile spermatozoa stuck to each other by antibodies with minimal involvement of other cells &/or debris.

- **Sperm Aggregation**: dead sperms stuck to other cells &/or debris rather than to each others.

- **N.B** 1. at least 10 fields should be examined ✗

2. appears:
   - clinically: small clumps in NL semen
   - M.C. stuck may be: Head to Head, Head to tail, tail to tail.

3. Agglutination is significant if > 10% of sperms are agglutinated.

abNL → > 10% stuck é each other

Immunobead Test / MAR Test → Do Immunological Tests.

if +ve

Search for the Cause ✓

e.g. infection
immunological problem.

NB: (1) Any borderline sperm → Counted as AbNL

(2) Teratozoospermia means:

- Normal forms < 30% WHO or < 14% KSCM
- TZI is > 1.5.

2 Indices → TZI (MAI)

(Teratozoospermia index = Multiple anomalies index)

= Total No of defects / No of AbNL forms

= (Σ no. of defects in all sperms) / (no. of all sperms)

If: 1 means: each AbNL sperm has one defect.

3 means: Each AbNL sperm has 3 defects.

NLLy: TZI < 1.5 & if → good prognosis

> 1.5 → significant ↓ in fertility.

& X MoI (Motile Oval Index)

Used to evaluate the 3 sperm parameters at the same time:

= Count x % of motility x % of oval sperms

So lower limits of MOI =

20 x 40% x 60% = 4.8 million sp. Imp.

Also SDI (sperm deformity index): No of defects / Total no of sperms

NLLy: upto 1.5

3 Anomalies

OAT (oligoasthenoteratozoosp.)

Def.: ↓↓ of 3 sperm Parameters:

- Count
- Motility
- Morphology

[Stress Pattern]

Occurs in any condition Associated with Testicular Stress i.e. any

- Varicocele
- Heat Exposure
- Systemic illness.

Examples → [Fever, irradiation.

Globozoospermia (Round head Synd)

* Sperms that show:

- By LM: Round Head
- By EM: defective Acrosome (Absent or small). < 2/3 head

Sterelizing sperm defects

= Globozoospermia + Immotile Cilia Synd. (Kartagener's)

Immunological tests include

Immunobead test

Polyacrylamide beads Coated with Anti human Ig's (IgG, IgA)

+

Suspension of (Sperms) (2ème)

(Centrifugation → remove seminal plasma)

↓

Comment on: ① % of Sperms bind to beads
② Pattern of Binding
③ Class of Abs.

• Test is +ve if ≥ 50% of Spermatozoa bind to Immunobead → Immunological infert.

Mixed Antiglobulin Reaction (MAR) test.

→ Latex Coated with anti Human (or Sheep RBC) Igs (IgG, IgA)

+

Fresh Semen Sample (No Centrifugation)

↓

+Ve test indicated by demonestration of Sperms attached to the Latex.

• the test ±:
- −Ve : if < 10% of Sperms attached to the Latex
- +Ve : if 10–90% of Sperms attached to Latex
- Strg +ve : > 90% ...

NB : the most specific is Immunobead test.

• others tests
- RIA
- Sperm immobilization Test

6 Non Sperm Cells. (Round Cells)

Def → Cells other than sperms that are present in semen → Collectively known as **Non Sperm cells** or **Round Cells**:

- ✓ Leukocytes
- ✓ Immature spermatogenic cells.
- Epithelial cells
- ✓ RBCs
- protozoa

To differentiate bet them

WBCs

All semen samples contain (WBCs)

- NL level < 1 million /ml or < 5 HPF (high power field)
- Excessive no → infection (Leukocyto-spermia = Leukospermia)
  - ↓ sperm quality (by ↑ Ros)
- Can be detected by:

1. Peroxidase test: depends on the presence of peroxidase Enz. in granules of (PNL)

disadv: Can't identify:

a. Activated WBCs ω released their grs.
b. WBCs that don't contain peroxidase.

Immature Spermatogenic Cells

- include:
  - Immature Spermatid.
  - Spermatocytes.
  - Spermatogonia.
- Significance:

Their Excessive presence indicate → defective spermatogenesis (shedding)

- Stain:
  - Leishman
  - PAS (or Acrascan)

2. Immunological method: using monoclonal Antibody to detect Leukocytes specific Ag (CD45). [Leukocyte pan markers]

Functions of The sperms

- Inside Male Genital Tract
  1. Sperm Motility
  2. Fertilizing ability
- Inside Female Genital Tract
  1. inside (Cx Ut Tubes)
  2. Sperm ovum interaction.

(Capicitation, Acrosome Reaction)

I. Function inside ♂ genital Tract

Sperm motility

1. Non motile testicular sperms are transformed into motile sperms inside the epididymis
2. Energy required for motility is derived from Mitochondrial ATP

(ATP $\xrightarrow[\text{enz.}]{\text{ATPase}}$ ADP $\longrightarrow$ energy release)

3. Mechanism of Motility:
   - ATPase enz. is Activated inside one half of the Axonemal ring $\longrightarrow$ The dynein arms will pull on the adjacent doublet microtubules of this side $\longrightarrow$ Sliding of doublet microtubules $\longrightarrow$ bending of tail in specific direction $\longrightarrow$ switch of the process

Sperm fertilizing Ability

- Testicular sperms have No Fertilizing ability but attain it inside the epididymis.

of { Activation / sliding / bending } to other side of the Axoneme → bending to the other direction.

Detailed Mechanism

- Dynein: is Mg dependant ATPase → driving force for motility.
- Doublet Microtubs. interact each other → sliding &
  - Central / Nexin / Spoke: → interact & Convert the tubular sliding
  - To "Flagellar Wave form"

- 4 elements responsible for initiation & maintenance of Motility: cAMP / Ca+ / protein phosphorylation* / Protein Kinase A** (PKA) (Key enzyme).
  - Both { PKA & protein Phosphorylation } is cAMP dependa

- other factors that help (in semen):
  - Zinc
  - Citric acid
  - Fructose
  - bicarbonate
  - PGs
  - Choline Compounds.

- The fibrous sheath of the tail is composed of Flagellar protein called outer dense fiber Protein (ODF) which is passive-elastic & rather stiff element that Transmit the Kinetic Energy from the axoneme to words the Junction of Flagellum & Sperm Head. & d.t bigger diameter at this point → Torque.

"سنك" ... Zinc: Concentrated in Large amount (75%) to ODF.

- Role of ROS: inNormal events: regulate { AR / Capacitation } → Controlled by balance bet it & Scavenger
  - if ↑↑ → oxidative Stress
    - effects: 1. Lipid Peroxidation 2. axonemal damage.
    - Source: NL & dysfun Sperm. WBCs
    - main Scavenger: SOD & GPX others { Vit C / Zinc / VIT E }

II. Function of the Sperm inside the Female Genital Tract.

1. inside < Cx / UT — < Penetration of Cx Mucus / Migration through the UT.

سم بيدن ٢ خطوات .

2. inside the tubes < Sperm Capicitation & Hyperactivation.

سم بيدن فيالوبيم .

3. Sperm ovum interaction:

سم بيدن ٦ خطوات .

So Sperm function inside the ♀ Genital Tract

عبارة عن رحلة الحيوان المنوي داخل "female genital tract"

وهذه الرحلة عبارة عن ٤ خطوة .

((Steps))

1. Rapid Transport

- immediately after ejaculation
- Takes: 10-20 mins.
- rapid Transport of sperms Inside the cervical canal helped by UT. Contraction during Coitus. (AG < motility + UT Contract)

usually: 200 - 500 mill Sperms are deposited in post. vaginal Fornix.

"To be protected from vaginal acidity"

↓

2. Sperm Colonization & reservior Formation

- Takes : upt 48 hrs.
- Colonization of Large No inside the Cervical Crypts → Formation of sperm reservior. (ensures : Constant release & ↑ chance of Fertilizat°).

3. Slow Release & Transport :

- Sequential release of sperms from the cervical reservior helped by UT. Contraction d.t PGs inside The Semen (secreted by SV).

4. Sperm Capicitation :

- decapicitating factors: Acquired from epididymis & SV to prevent "premature capicitat°".
- "NO" may play role. (دور محتمل)

→ يحدث في UT / Tubes

def: Changes that occur for the sperms after separation from the Seminal plasma (which normally contain decapicitating factors that prevent Fertilizat°) during Transport through the UT & Tubes.. so it will be Capable of Fertilization.

- requires 3-4 hrs. during which sperm memb. shows the following changes:
  1. removal of Glycoprotein Coat & Semin. plasma proteins from the plasma memb. over ! acrosom
  2. Destabilization & ↑ Permeability.
  3. Efflux of cholesterol.
  4. Influx of: Na^+, K^+, Ca^{++}, O_2 & Glucose.

5. Sperm Hyperactivation

3 Changes:
- vigrous ↑ in amplitude of tail bending.
- ↓ Linear (progressive motility)
- attainment of Circular motility.

6 & 7. Penetration of
- Cumulus Oophorus : depend on Hyperactivity + Hyaluronidase enz.
- Corona Radiata : depend on esterase enz. (Corona penetrating enz.)

↓

8. Zona binding : d.t interaction bet. specific sperm receptors & specific ZP protein (ZP3)

↓

9. Acrosome Reaction (Zona Penetration). (AR).

def. process of Activation of proacrosin to Acrosin followed by Zona Penetration.

← Mechanism

- The Acrosomal Cap has 2 Layers:
  - outer Acrosomal memb (OAM)
  - Inner Acrosomal memb. (IAM)

  ↓ Contain : inactivated enz called Proacrosin.
- Steps of Activation:
  1. lifting or elevation of plasma memb. ⇒ outer acrosomal Membrane
  2. dispersion of OAM
  3. Formation of multiple fusion points bet. 2 memb. → Fusion Pores
  4. dispersion of Fused membs. as a vesicles
  5. Exposure of IAM → Proacrosin → Acrosin → Zona Penetration ✓

ZP: Penetrated by Acrosin enz.

↓

10. Zona penetration

↓

11. Fusion bet. Sperm membrane & oocyte Membrane

↓

12. Cortical Reaction (Zona reaction)

exocytosis of cortical granules → Cortical reaction → Hardening of Zona → Polyspermy Blocking (-- of Zona penetration by other Sperms).

↓

13. Formation of Male Pronucleus [Chromatin decondensation]

↓

14. Nuclear Fusion : DNA of sperm + DNA of ovum → Zygote.

NB

- The sperm pass by movements of their tails through the Cx. Canal but passage through the UT & Tube is assisted by muscular contractions of them.

So: at Cervix → Sperm motility

after Cx:
- Sperm motility
- Myometrial Contract^
- Mesalpinx
- Female orgasm.

- Isthmus of F. tube: is main functional reservior where sperm remains until ovulation.

- Survival time for:
  - Sperm inside female Tract: upto 48 hr.
  - Oocyte: 12-24 hr.

- Role of Cx during sperm transport:
  1. Sperm reservior
  2. Leucocytic reaction: Cx will ↑↑ No of WBCs in mucous → Prevent ig.
  3. mid Cycle Mucus → allow sperm Penetrat^
  4. Immune Suppressive role.
  5. energy to the sperm
  6. Sperm select^: only motile & NL Can Penetrate (See KSCM

- For Staining of Capicilation & Acrosome React^ →

"Chlortetracycline Staining"

- Factors affecting Zona binding & Acrosome reaction

① Sperm — morphology, motility, Concentration in Insiminat^ media.

Specially : NL Acrosome.
(عشان كده اهم حاجه ع Morphology Assess. is Acrosome Assess.)

Inducers of AR.

② Ca + Ionophore A23187

③ Human follicular fluid

④ Progesterone

⑥ Native Human ZP (most efficient factor for induct^ of AR).

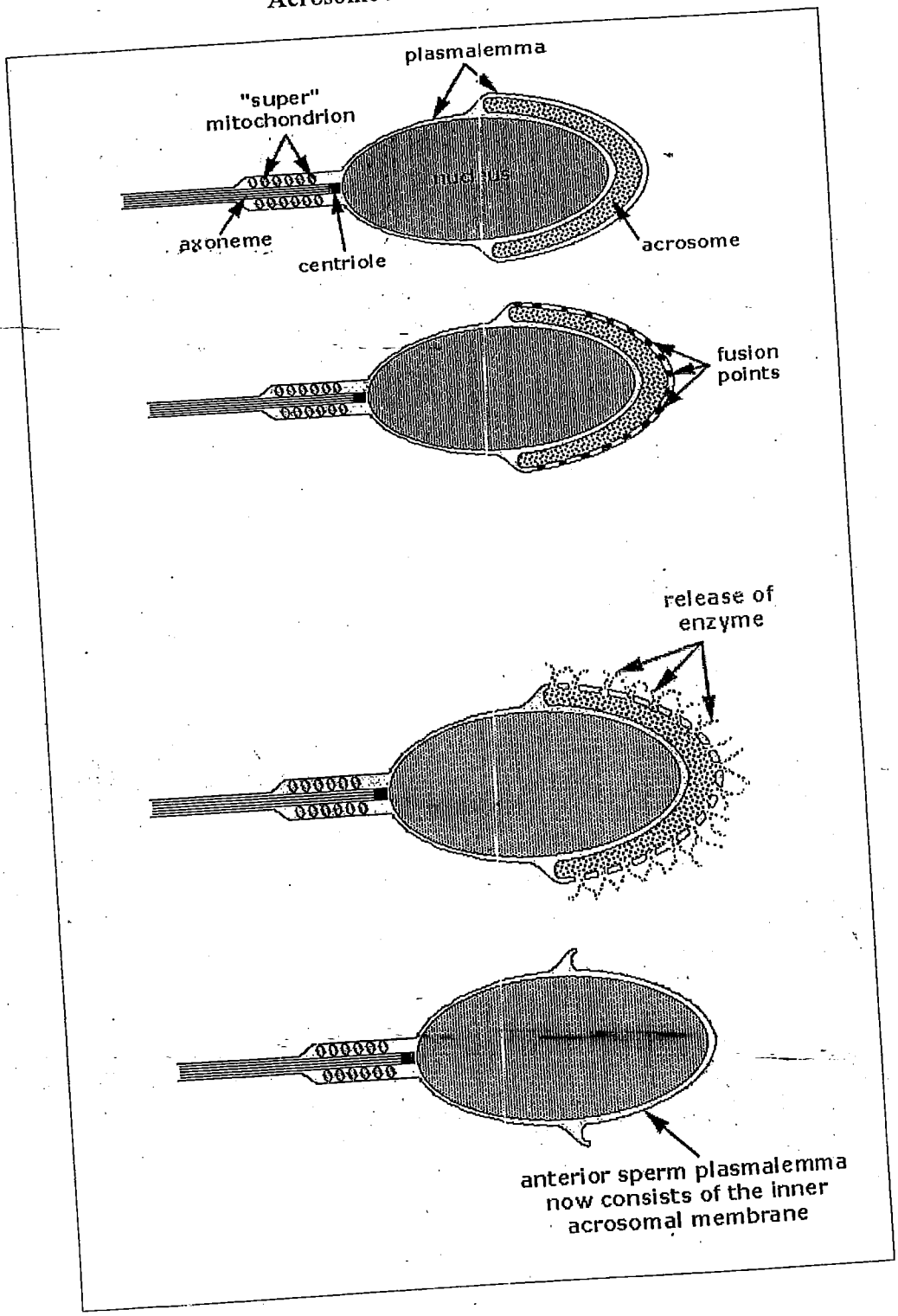
- NB. AbnL AR: may be found in 5% of infertile men.

5% → prematurity

50% → insufficiency

- Pentoxifylline: doesn't ++ AR but ↑ sensitivity of Sperm to Ca+A23178. Ionophore.

Acrosome Reaction



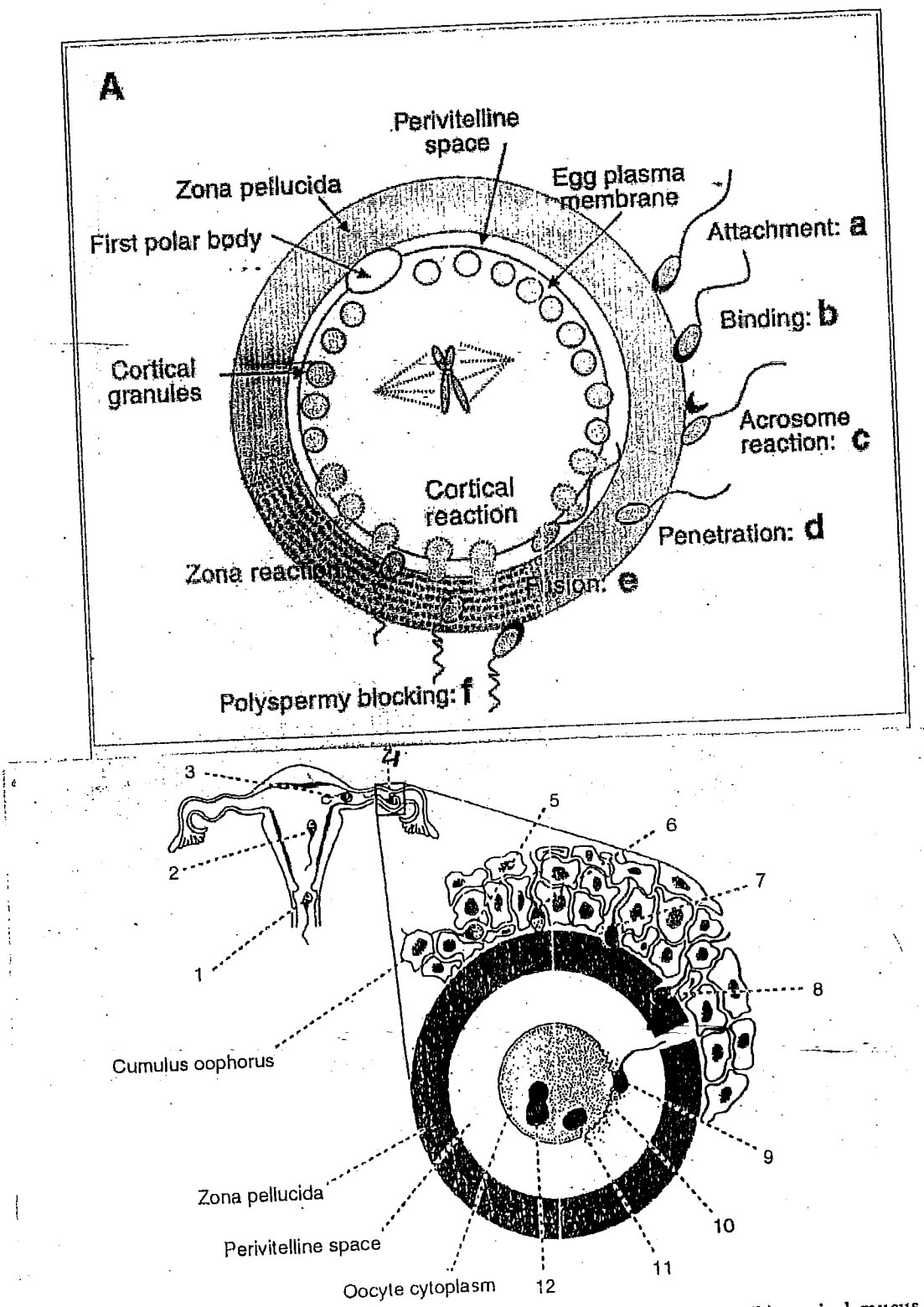
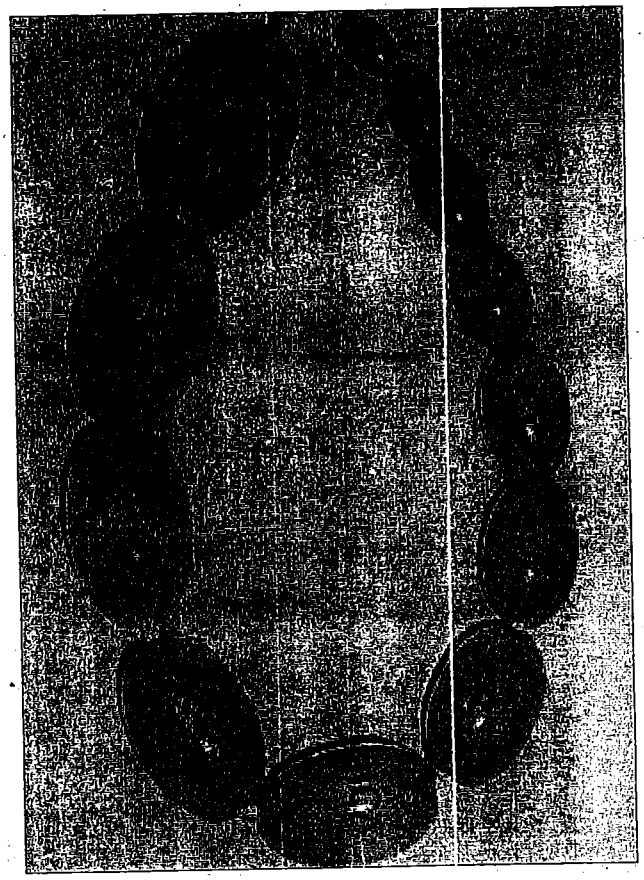
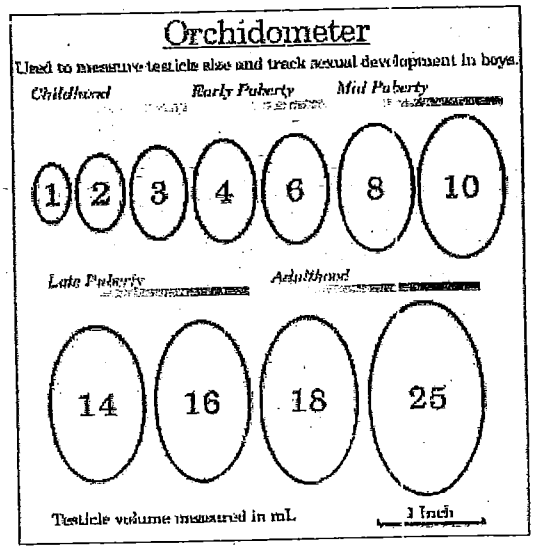
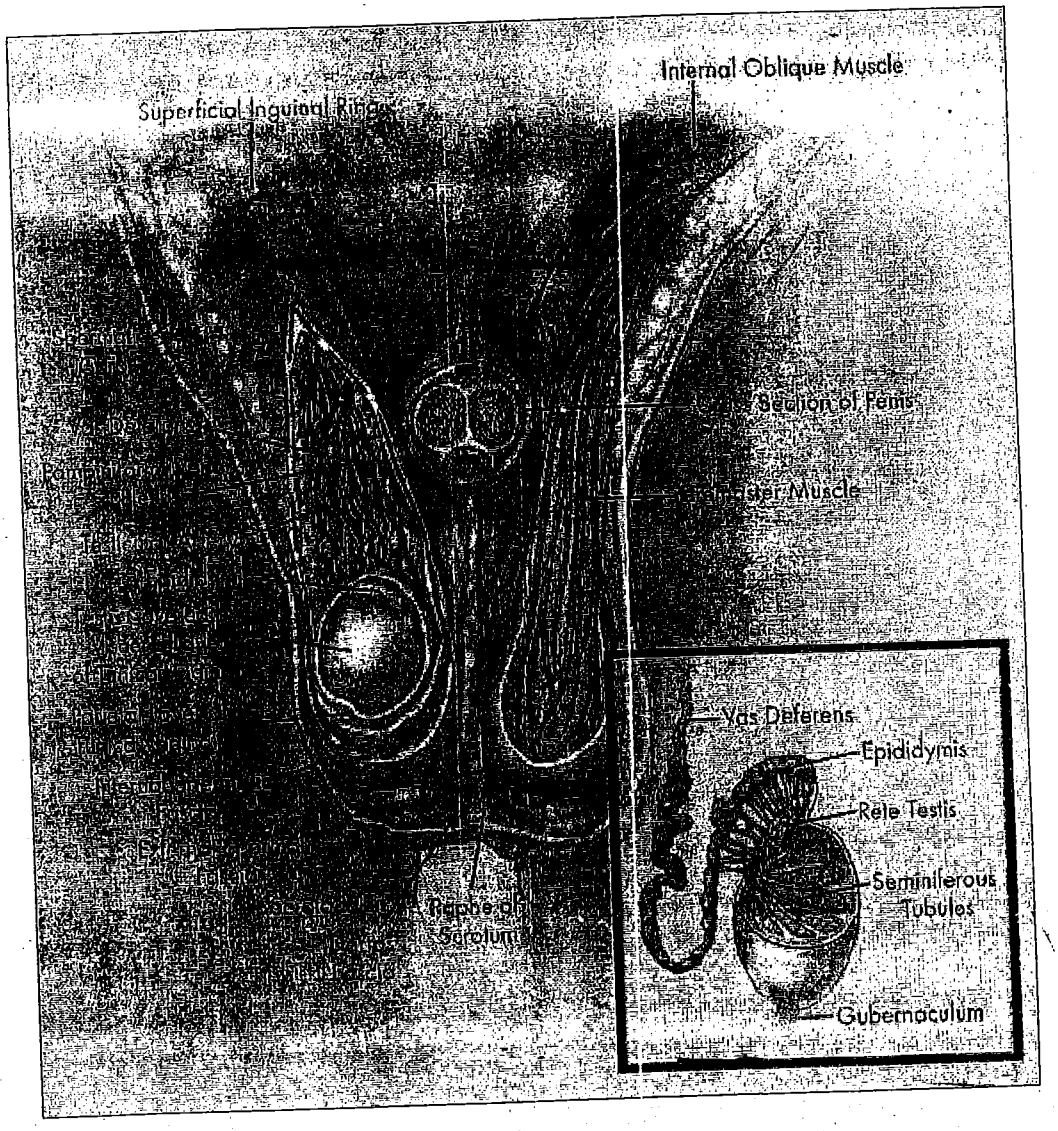


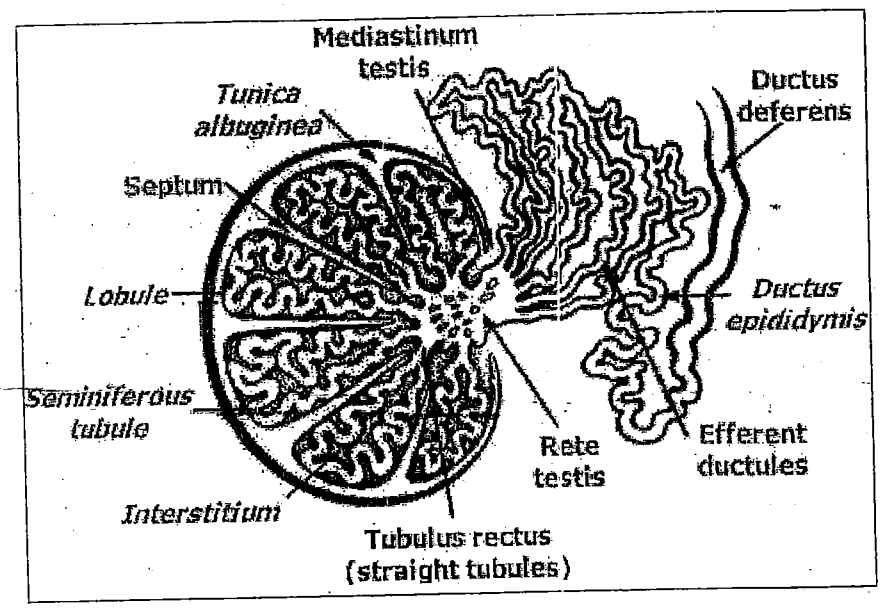
Fig. (22): Process of fertilization (1a) deposition of normal spermatozoa; (b) cervical mucus penetration; (2) sperm migration through the uterus; (3) sperm capacitation and hyperactivation in the fallopian tube; (4) an enlargement of spermatozoa-oocyte interaction (5) sperm penetration of cumulus oophorus; (6) sperm binding to zona pellucida; (7) acrosome reaction; (8) sperm penetration of zona pellucida; (9) sperm fusion with oocyte plasma membrane; (10) cortical granule exocytosis; (11) nuclear decondensation; (12) nuclear fusion (*Tripp and Gagnon, 1997*).

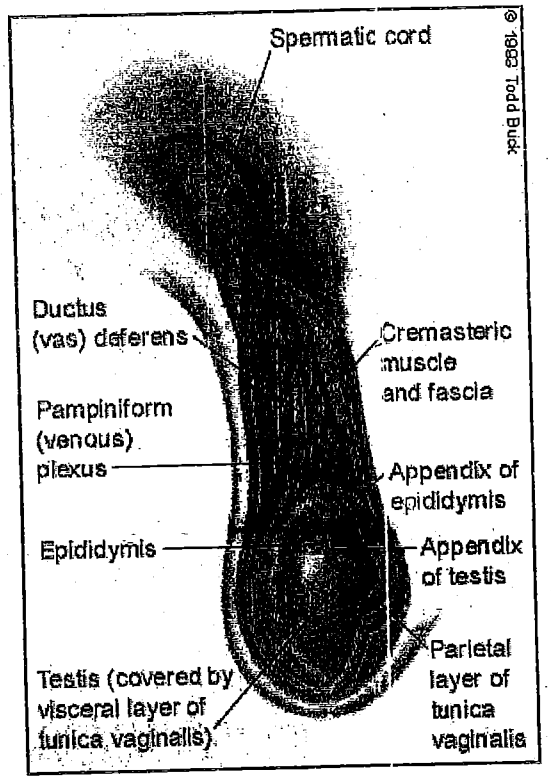
Prader orchidometer

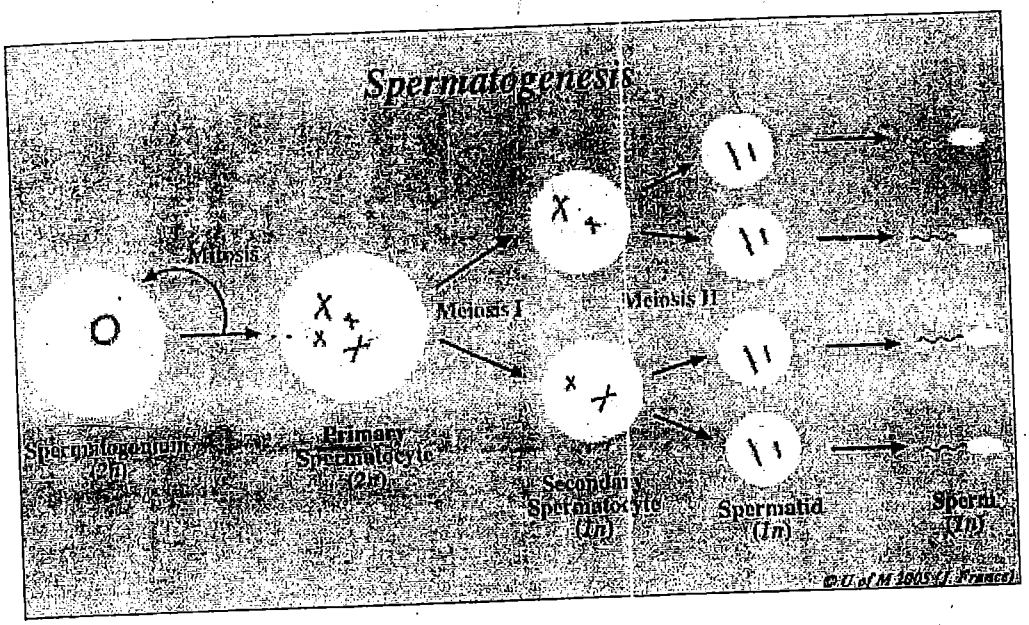


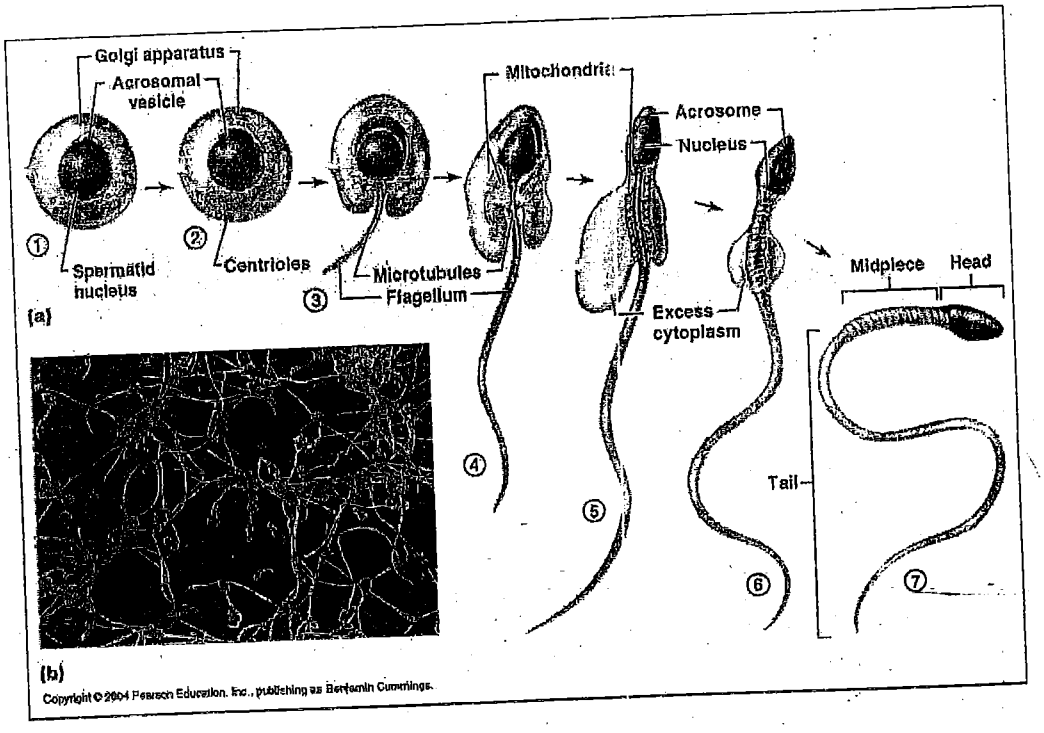


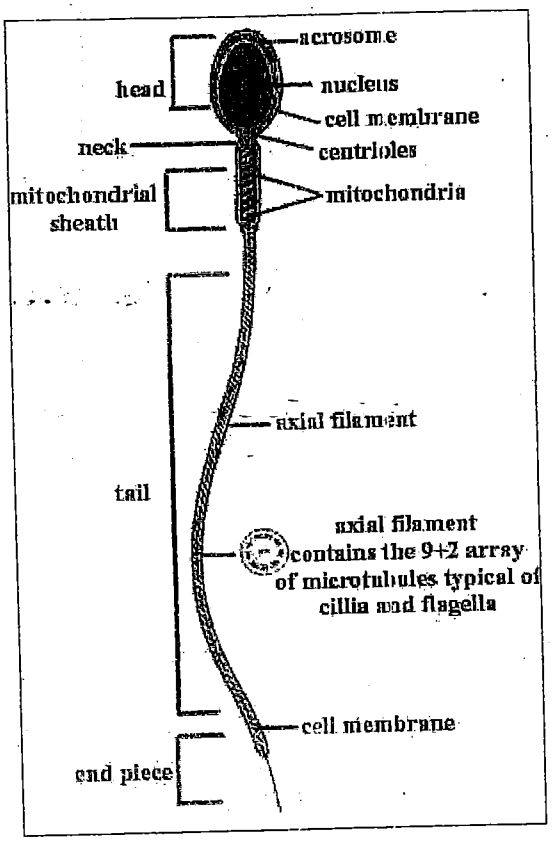

Internal Oblique Muscle
Superficial Inguinal Ring
Section of Femur
Raphe of Scrotum
Vas Deferens
Epididymis
Rete Testis
Seminiferous Tubules
Gubernaculum

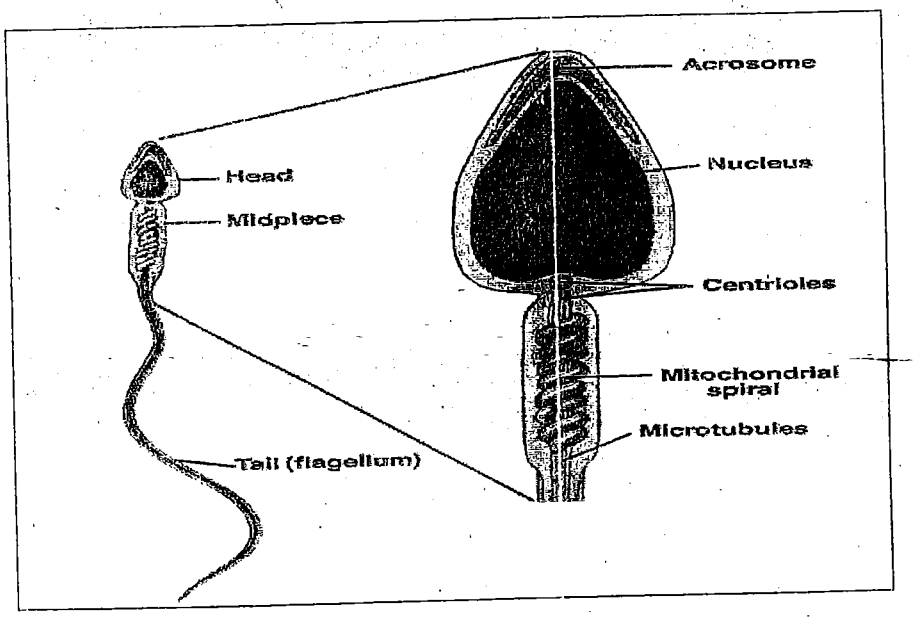

Mediastinum testis
Tunica albuginea
Septum
Lobule
Seminiferous tubule
Interstitium
Tubulus rectus (straight tubules)
Rete testis
Efferent ductules
Ductus epididymis
Ductus deferens


Spermatic cord
© 1952 Todd Buck
Ductus (vas) deferens
Pampiniform (venous) plexus
Epididymis
Testis (covered by visceral layer of tunica vaginalis)
Cremasteric muscle and fascia
Appendix of epididymis
Appendix of testis
Parietal layer of tunica vaginalis

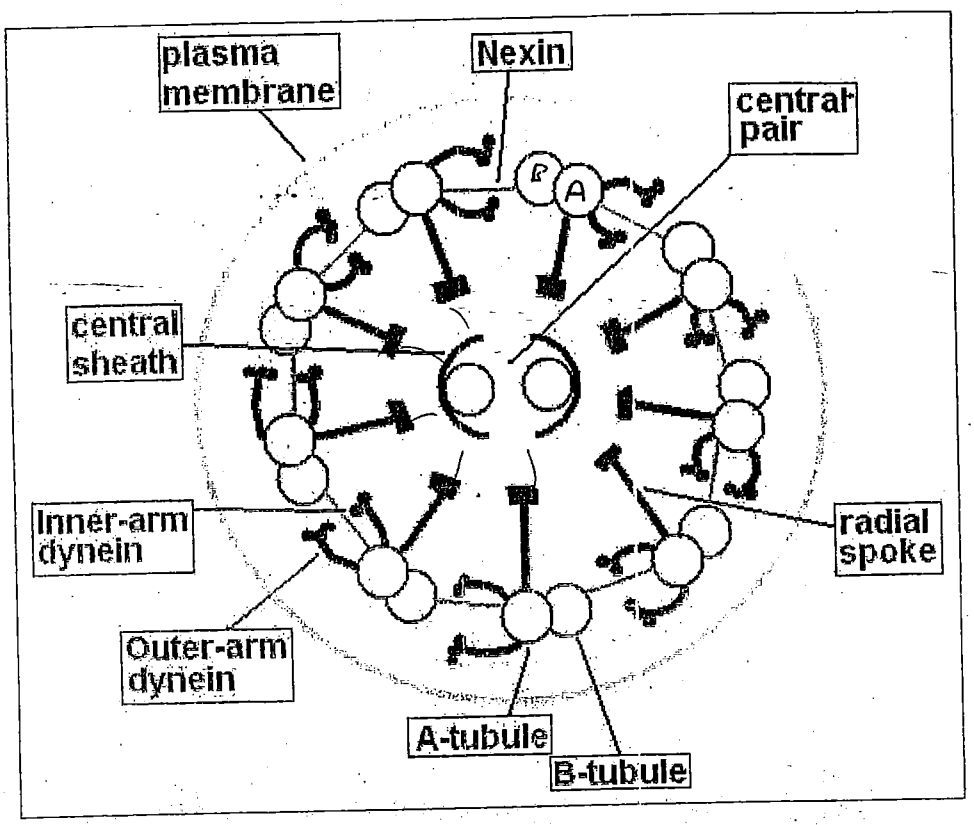

Spermatogenesis
Mitosis
Meiosis I
Meiosis II
Spermatogonium
(2n)
Primary
Spermatocyte
(2n)
Secondary
Spermatocyte
(1n)
Spermatid
(1n)
Sperm
(1n)
© U of M 2005 / J. Frasch

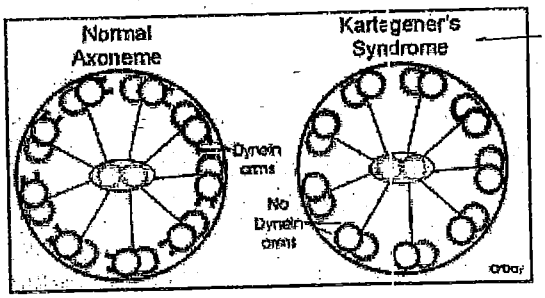

Golgi apparatus
Acrosomal vesicle
Mitochondria
Acrosome
Nucleus
Spermatid nucleus
Centrioles
Microtubules
Flagellum
Excess cytoplasm
Midpiece
Head
Tail
(a)
(b)
Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.


acrosome
head
nucleus
cell membrane
neck
centrioles
mitochondrial sheath
mitochondria
axial filament
tail
axial filament contains the 9+2 array of microtubules typical of cilia and flagella
cell membrane
end piece


Acrosome
Head
Nucleus
Midpiece
Centrioles
Mitochondrial spiral
Microtubules
Tail (flagellum)

Axoneme structure



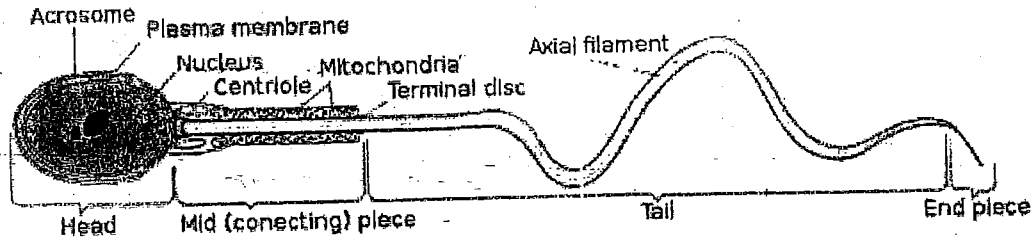


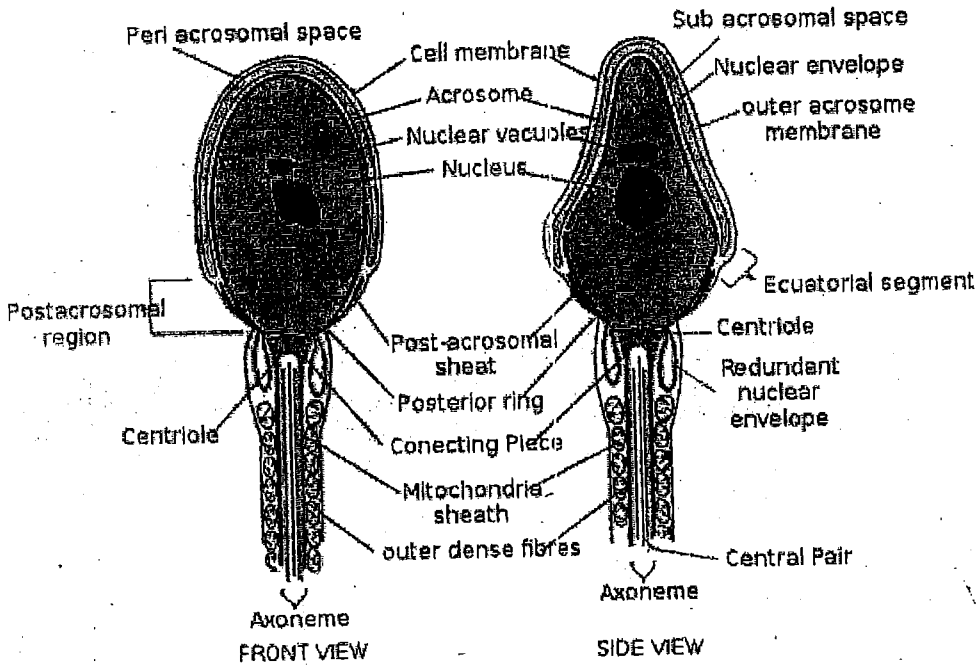
No Nexins

No dynine arm

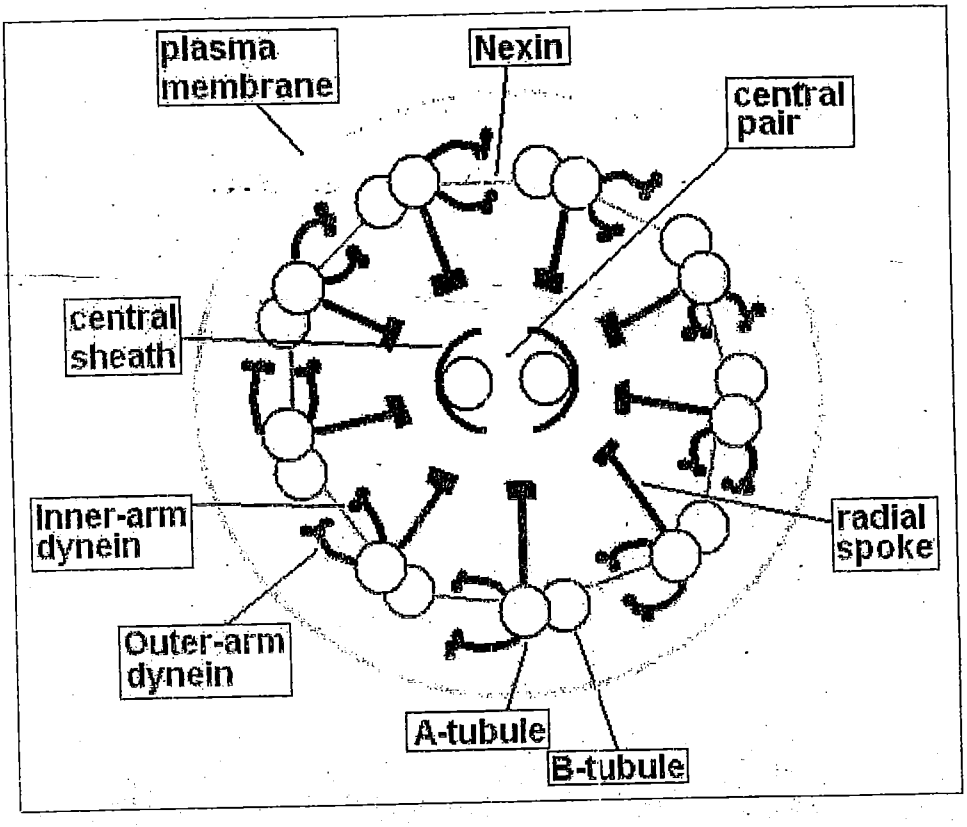
9+0 synd = No 2Centrl
Single Microtub.

Sperm structure



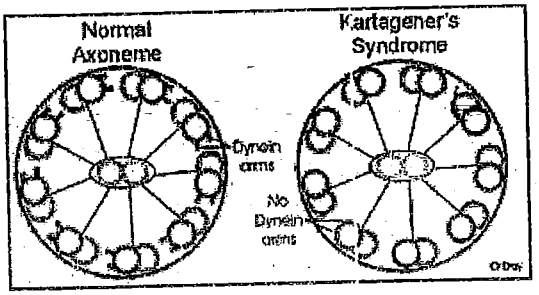


Axoneme structure



2 Abnormalities

1. Kartagener's Synd = Absent dynein arm & Nexins



2. 9+0 Synd : Absent Central Single Microtubule

physiology of the Testis

1. structure ↓ Testis is suspended by The spermatic Cord inside The Scrotum. Testis > SP. Cord > Scrotum

2. funct ↓ Later on.

3. Regulat & Control of funct ↓ Later on.

(structure)

Testis

1. Structure & anatomy.
2. Coverings.
3. Vs.
4. Histology.

Spermatic Cord ↓ Contains:

- 3 facial Layers
- 3 vascular structures
- 3 non "

Scrotum ↓ Contains 3 Layers

- Skin
- facia
- dartos m.

---

1. Testicular anatomy & structure

it has

- 3 dimensions (SPB) &
- 3 Coverings (3 Tunica)
- 3 intratesticular ducts (TRE).

oval

- dimensions: L 4.5 (3.5 - 5.5) X W 3 X 3 (A.P.D)
- Size — Volume 15 - 25 ml (size) < orchidometer, L, W (spiral Caliber), U/S — $W^2 \times L$
- axis & relation → (ch. 24) — vertically in scrotum
- Inner structure: The inner aspect of Tuni

NB Testicular L < 3 → Hypogonadism

Clinical Anatomy

L = 35-55
W = 25-35
Height = 15-25

Volume = LXWXHX 0.5-2

Endocrinology

Orchidometer

⇒ Testicular Volume (Normal)

- Prepubertal 1-3 ml
- Pubertal 4 ml
- Adult 12-25. → 0.8 Tubular, 15 Interstitial

Test. Size : 1st Sign of puberty

Macroorchidism
- MR
- fragile X Synd.

① Interstitial : 15% of Test Vol → 10-20%
occupied by Leydig

② Tubular Comparts : 60-80% of Test. Vol.
- Contains < 2 Germ Cells. < 2 Sertoli Cells < P

Albuginea gives many septae. That divided The testis from the ant. aspect to the post. no. These septae then converge at posterior asp. of testis to form a mass of fibrous tissue (1) Known as mediastinum testis which acts as a supporting structures to the testicular vessels & ducts That pass through it.

So that the parenchyma of the testis is divided by these septae into 300 lobules each lobule contain (1-3) Seminiferous tubules (Tubular Component). which are Convoluted

these seminiferous tubules: (3)

(1) Surr. by (3) Layers: Collagen, Myoid (Myoid Cells), Adventitial C. (Fibroblasts)

(2) Contain → 2 Cells: Germ, Sertoli — Rest on B.M.

(3) Each Convoluted seminiferous tubule ends in 2 "Straight Tubuli Recta (T)" which enter the Mediastinum testis forming Rete testis (R). Then from → the efferent ductules (10-19) enter the head of epididymis forming epididymal lobules that coalesce to form a single epididymal duct

· each Seminiferous tubules Composed of: (أجزاء)
  · B.M on it rests:
  · Germ cells.
  · Sertoli cell.

· Germ cells: These cells rests on the B.M & give rise to the ~~The~~ Spermatogenic cells that include different types of cells arranged in an organized pattern of maturation from the most primitive type of cells on! B.M to! most-advanced types of cells near the lumen of Seminiferous tubules.

· These cells include: (from below to up, sequental Maturation).

1. Spermatogonia → (2) types
2. primary Spermatocytes → (4) phases
3. 2ry Spermatocytes.
4. Spermatids → (6) types.
5. Mature sperm.

No of Germ cells = 13

· Spermatogonia include (2) type: A, B

→ AL → Type A Long → inactive
→ AD → type A dark → inactive
→ AP → type A pale → active (give rise to)
→ Type B spermatogonia.

↓

Primary Spermatocytes

4. Primary Spermatocytes are (4) phases:

- P-L : Pre/Leptotene
- L : Leptotene
- Z : Zygotene
- P : Pachytene → 2ry Spermatocytes

Short Lived & difficult to be identified in Histological sections.

- Spermatids:- give rise to

(6) types:

- Sa, Sb1, Sb2, Sc, Sd1, Sd2.

(Then) Spermatids are undergo Morphological changes into the mature sperms.

Sertoli Cells = Sustentacular (or) Supporting cells. (Nurse cell of testis) ≡ Mother cells.

Tall cells, e irregular nuclei, rest on the B.M of the tubules & send their prolonged cytoplasmic projections toward the lumen of the tubules.

Sertoli cells make 2 relationships:

① Sertoli cell - Sertoli cell relationship.
② " " - Germ cell "

Sertoli Cell - Sertoli Cell relationship: Tight Junctional Complexes exist bet. the adjacent sertoli cells → formation of blood testicular barrier .. ♂ is (not) formed before puberty

This barrier divides the seminiferous tubules into 2 compartments ① basal Compartment ♂ Contains the early stages of Germ Cells (← spermatogonia ← young spermatocytes.

② Adluminal Compartment ♂ contains the late stages of Spermatogenesis (mature spermatocytes & spermatids)

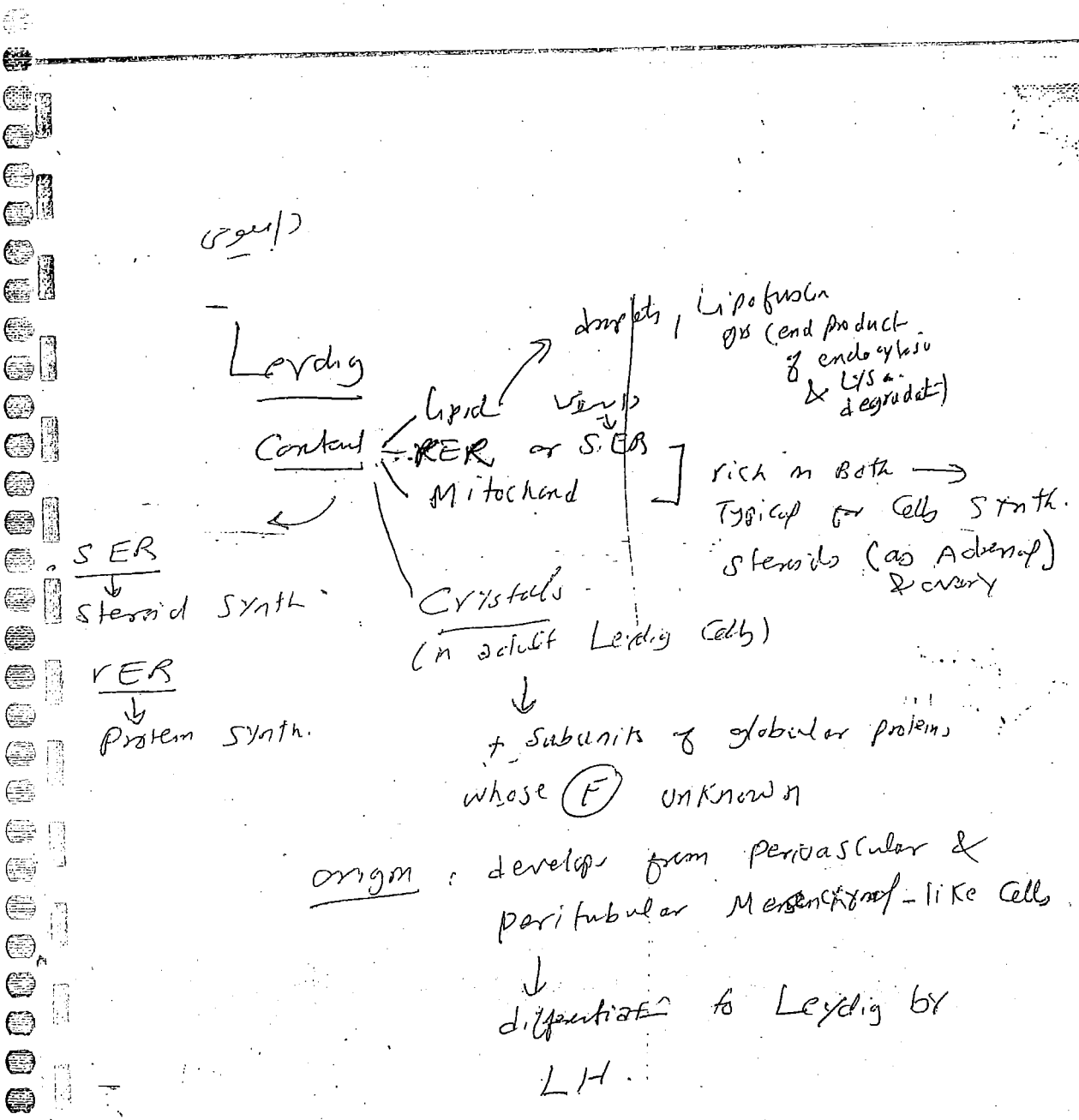
(F) ← Metabolic / protective

The barrier has both metabolic functions (by Controlling the passage of materials through it) & Protective Immunological functions (by prevention of passage of the antigenic sperms into the blood & Lymph Vs ♂ → Anti-sperm antibodies response. (Immunity)

Sertoli Cell - germ cell relationship:

There is a physical Contact bet. the 2 cells, ♂ may help in the organized pattern transport of the germ cells in a toward the lumen of the tubule by the specialized changes in the configuration of Sertoli cells

Finally, the late spermatids are found inside recesses (invaginations) of the apical portions of the Sertoli cells. They attain their


Leydig
Content
Lipid droplets / Lipofuscin
or (end product of endocytosis & Lys. degradation)
RER or SER
Mitochond
rich in Both
Typical for Cells Synth. steroids (as Adrenal & ovary)
SER
steroid synth.
RER
protein synth.
Crystals
(in adult Leydig cells)
± Subunits of globular proteins
whose (F) unknown
origin : develop from perivascular & peritubular Mesenchymal-like cells
differentiate to Leydig by
LH

⑥ Maturation by losing their Cytoplasm that's phagocytosed by Sertoli cells, finally they are released by the process of (Spermiation) from the recesses of Sertoli cells.

Interstitial Compartment of testis:

The space bet. the tubules .. Contain:

1. Blood & Lymph Vs / NS.
2. fibroblastic supporting cells.
3. Macrophages
4. Leydig cells.

(BVs & Lymph, fibroblast & Mac, Leydig)

- Present in clusters
- Account for 5-12% of the testicular Vol.
- Each cell has around nucleous & prominent Mitochondria, S.E.R, Reinke Crystals & lipid droplets.

Shape: Polygonal. Rough endoplasmic reticulum Extensive
Nuc. Round & Vesic. Cytop. Eos.
Contain: SER, lipid droplets, Reinke Crystals (Eosinophilic inclusion), Lipo-

Fxn ⇒ secretion of testosterone

Coverings of the testis: "من برا لجوا"

1. Tunica Vaginalis: formed of outer parietal Layer & inner Visceral Layer. the space bet. The 2 Layers Contains some clear fluid that may be Pathologically (↑) in Cases of Hydrocele.

("2 Layers" ← outer parietal, inner visceral)

2. Tunica Albuginea: Tough fibrous Layer (formed of Collagen + Smooth m. cells that give the testicular Capsule a contractile activity & helps to regulate flow of Testicular fluids.

(Contract act ⇒ helps)

Functions of Sertoli Cells = mother or nurse cells = sustentacular cells = supporting cells

(Support of Spermatogenesis)

Support & Regulat. Sp.

Regulat. (3):
- physical regulat.
- Chemical regulat.
- Immunological regulat.

① physical reg. of spermatog. — Anchoring / Transport / release of germ cells.

The physical contact bet. Sertoli cells & the developing germ cells (anch. / transp. / release), help the propagation of germ cells from the base toward the lumen of seminiferous tubules, finally, the mature sperms are released from (recesses = invaginations) in the cytoplasm of Sertoli cells into the seminiferous tubular lumen by the process of "spermiat."

(Anchoring → Transport → release by spermiat.).

All these processes of physical contact bet. Sertoli & germ cells are dependant on 3 groups of specific structures of Sertoli cells as following:

1. Cell skeleton structures: e.g. actin filaments & micro-tubules that help morphological changes of Sertoli cells & their anchoring of germ cells.
2. Cell surface structure: Cell junctions → Blood testis barrier; invading processes. Examples are: Cellular juncts (that form blood testis barrier) & invading processes (that anchor the germ cells to Sertoli cells).
3. Cell adhesion molecules: examples include Cadherins & testins that help transport of germ cells along the Sertoli cells.
   - ① Cadherins
   - ② Testins

   → Transpo.

Free iron

- Needed for Cell division

Transferrin in Serum :

Correlate ē Seminal Parameters including - Sperm density

- SPARC
  - involved in Ca^+ Transport
  - regulate [TGF-β → differentiate & Sertoli
    → Matrix-Metallo-proteases (involved in cell adhesion.)
    ↓
    disrupt Collagen → disrupt BTB

- CRAB
  - Retinol (Vit A):
    - maintains B.T.B
    - ++T effect on Sertoli
    - helps adhesion of Spermatogenic N. S

Sertoli Cell Reg. & Testis development

② Chemical regulat of Spermatogenesis

The Secretory Products of Sertoli Cells that Can regulate Spermatogenesis can be divided into ③:

↓ Transport & binding Protein

examples:

① ABP (Androg. binding ptn): it Transports androgens To germ. cells (androg required for spermatog.)

② Glutamyl Trans-Peptidase for a.a.

③ Retinol binding ptn for V.A (RBP)

④ Transferrin for Iron.

⑤ Ceruplasmin for Copper

⑥ SPARC (secreted protein that is acidic & rich in Cystine ... for Ca^{+} Transport into germ cells ... is needed for Spermatogenesis.

ABP, RBP, SPARC + ? (Fe, Cu)

ABP, RBP: Androg
Fe, Cu: act

↓ Proteases & anti

- Sert. → Protease
- Germ → anti

② Protease induces Controlled proteolysis (is needed for Sertoli Cell remodelling & Germ Cells movement & spermiat...)

③ Anti proteases: help to protect the cells from some effects of Proteases.

Proteases ↓ Anti-proteases ↓

↓ Growth factors

MIS, Activin, Inhibin, IGF, IL1, IL2

These factors Control Cellular Structure & Functions

How ? by 2 Mechanisms

Examples of These factors

① MIS (Mullerian Inhibitory Subst)

↓

has 2 effects:

- Prenatal on Mullerian ducts.
- Postnatal: on Spermatogenesis ✓

② Inhibin & Activin:

- Inhibin: Secreted from Sertoli in response to FSH & → ⊣ FSH & Spermatogenesis.
- Activin: it has antagonistic action to Inhibin.

③ IGF-1 (insuline-like growth factor 1) ✗ IGF: Transforming Growth factor

④ Cytokines (IL1 & IL2)

How these growth factors: Control Cellular functions

Structures: → By ② Mechanisms

Systemic Control

① Neurologic Control: through Nervous System.

② Endocrine Controls Through Hormones that reach via Blood.

Local Control.

① Autocrine Control (Cells) produce Chemical Substances that act on the same secreting cells.

② Paracrine Control: The Chemical substances that are secreted act on adjacent cells.

③ Immunological Protection: → Blood testicular barrier of spermatogenesis.

This function of Sertoli cells depends on the blood testicular barrier & TGF-β (Transforming growth factor-(β)) that has immune protective function for sperms both inside the testis & inside the vagina as it is activated in acidic PH of Vagina.

Physical By.

- anchoring
- Transport
- Release

Chemical

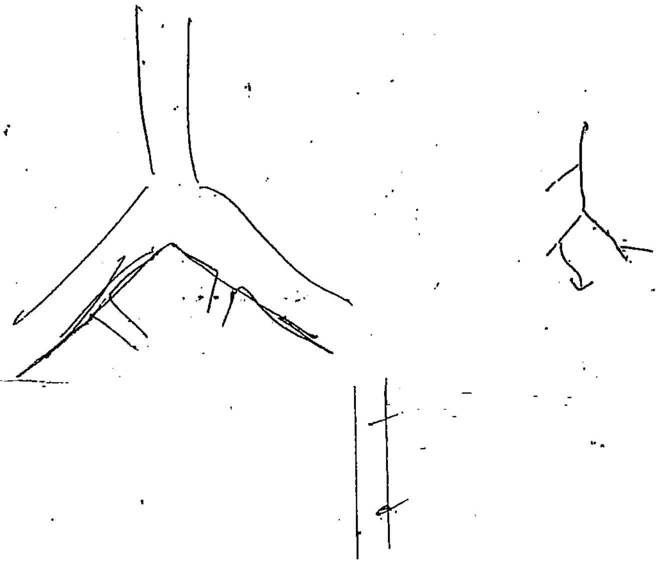
- ABP
- RBP
- Transf. Fe
- Cerul. Copper
- SPARC

- Protease & anti n
- Growth factors

Immunological

- MHC ✗
- Activin
- Inhibin
- IL1
- IL2
- IGF-1
- TGF1

Thymo



Blood supply (TVC)

1. من Aorta
   - below Renal a.
   - (Testicular)
   - Mediast. (Net work)
   - Supply < Test, upper epid.

2. واحد من int. iliac
   - Vasal a
   - from sup. vesical
   - tail by network that supplies Test.
   - VW, lower epi.

3. واحد من Ext. iliac
   - Cremastic
   - from Inf. epig.
   - T.V. By anast. with other aa.

TVC

TVC

V A V

③ Tunica Vasculosa: (The inner Layer of T. Albuginea)

- is highly vascular
- Accordingly, Meticulous surgical hemostasis should be done during closure of T. Albug. during open testicular biopsy to avoid intra-testicular Hge

---

Testicular Vs < Arteries / Veins / Lymphatics >

Testicular arteries: → ③

1- Testicular art. (internal spermatic a.)
- the main a. of the testis
- br. from the Aorta just below the renal a.
- Crosses the ureter supplying it, Then enter the inguinal canal in the spermatic cord
- Supplies the testis ē convoluted network of vs through the mediastinum testis.
- gives br. to upper part of epididymis.

2- Vasal (deferential a.)
- br. from iliac inf. vesical a.
- Passes inclose contact ē Vas supplying it.
- tail of epididymis by supplying the Testis by Capillary Network

3- Cremasteric a. (ext. spermatic a.)
- br. from inf. epigastric a.
- Passes along the sheath of Sp. Cord
- T. Vaginalis by anastomosing ē other arteries.

Testis → upper epid.

Vas → end at tail & epid. supplies it

(8)

Surgical important Points:

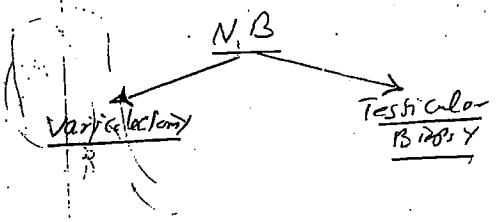
① Varicocelectomy & Testicular artery:

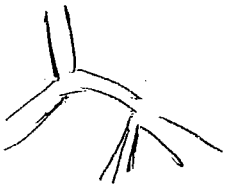
The rich arterial supply to the testis through 3 different arteries ē rich anastomosis bet. them helps to prevent testicular ~~atrophy~~ atrophy if The testicular a. was accidentally injured during Varicocelectomy. However this is not physiological to preserve it during the operation.

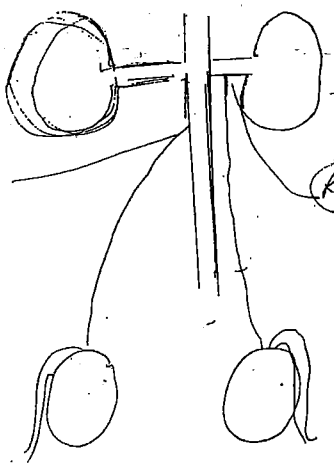
② Testicular Biopsy & Testicular a:

The recent performance of multiple sites (biopsy) may be associated ē accidental inj. of the testicular a. branches inside the testis (see testic. Biopsy)... This may be prevented by performing the least possible incisions during the procedure that should be also in the last Vascular area ū are the medial & lateral upper poles of the testis.

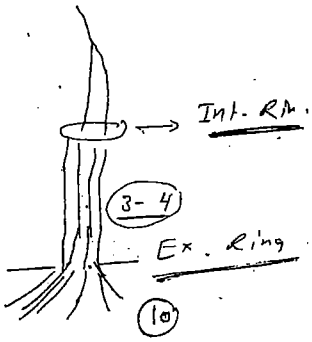
(11)







acute angle



Papiniform plexus

emerge from back of (testis), Recieve Tributaries from epid. → form Convoluted plexus (main main of Cord)

ascend in Cord, infront of Vas

at ext. Ring → unite → (3-4 veins) at

int. Ring [2 veins] → ascend → (Single Vein)

Testicular veins → (3) groups.

(1) Ant. groups (Testicular veins)

They are 10 veins that anastmose to form the pampiniform plexus of veins ⓦ is closely associated ē the testicular a.. The brs. are ↓↓ in No gradually till they pass through the ext. inguinal ring where they become the single testicular vein.

- The left testic. (spermatic) vein —drains into→ left renal vein Rt angle
- the R. " " " —"→ IVC. acute angle

(2) Middle groups (Vasal or deferential vein)

The vasal veins & the funicular veins that accompany ! drain the vas & epididymis

Vas deferens → drain into (Prostatic plexus) → (internal iliac)

& (Vesical plexus.)

(3) Posterior group (Cremasteric veins):

The cremasteric veins become separated from the spermatic cord at the ext. inguinal ring & drain into → (inferior epigasteric) vein — (IVC) Ex. iliac

Surgically important points:

- There is anastmotic sites bet. the right & left venous systems at the level of inguinal region,

(test.) ant → ..... & prostatic plexus → int. iliac
(vasal) mid →
..... post → inf. epig. → ext. iliac

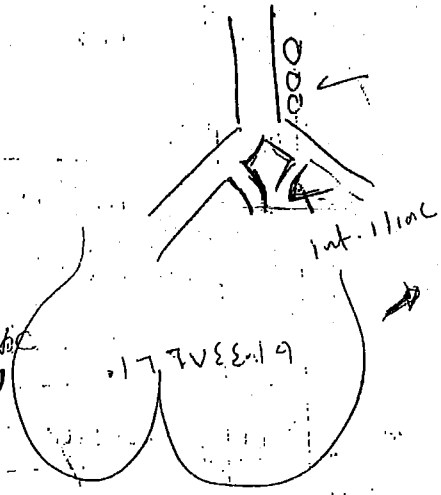
So that: Varicocelectomy should be done at! inguinal or supra inguinal level (to) prevent The abnormal Venous reflux in both R (t) & Left Sides.

Testicular Lymphatics:

Intra testicular Lymph ducts originate in the testicular interstitium & ascend inside the spermatic cord to drain finally into Para aortic L.Ns at Lumbar region (In the testis develop during the intrauterine Period).

So that testicular malignancy will give rise to paraortic metastasis inside the abd. & not to! inguinal region.

① Testis → Paraortic
② Covering → inf. iliac L.Ns
③ Scrotum → inguinal.



Nerve supply (Vis. Somatic supply)

Sympth & Parasymp

T10, 11

The Spermatic Card:

③ → Facial Layers. ③
Vascular Structures. ③
non " ". ③

A Facial Layers ③: The Cord includes 3 Facial layers that are derived from the ③ Layers of the ant. abd. wall:

① ext. spermatic facia: → derived from ext. oblique aponeurosis.

② Cremasteric ms & facia: derived from Int. oblique & Transversus abdominis mscls.

③ Internal spermatic facia: derived from Transversalis facia.

B Vascular Structures ③: 3 aas, 3 Veins & Lymphatic

C Non vascular structures ③: Vas. Cremasteric m. Testicular nerves:

Super spermatic Nerve derived from < Renal / Inter mesentric Plexus

So UT disease → Test. pain

arises from thoracic (10 & 11) & may be from the first Lumbar & pass ē the testicular artery Through the inguinal Canal then inside / Sp. Cord supplying the tunica albuginea ē autonomic pain receptors while testicular parenchyma itself devoid of pain receptors.

* Clinically important Points:
d.t high origin of Testicular nerves, the Testicular pain may be referred to / upper Abd

No Somatic / Nerves mainly restricted to small intersts

Function of the testis

testis → 2
- spermatogenesis ← Germ cells, Sertoli cells
- Steroidogenesis → Leydig cells

* Spermatogenesis occur by Germ cells while support of spermatogenesis occurs by Sertoli cells.

* spermatogenesis → 3 phases
  - prolif. phase
  - Meiotic
  - spermiogenesis

* **prolif. phase** : عبارة عن

stem cells (double no of chromosom) (spermatogonia) → prolif (mitotic) → Type (A) spermatogonia → A (inactive) / A (Active) → Type B spermatogonia → 1ry spermatocytes (2N)

* **Meiotic phase** : عبارة عن

1ry spermatocytes (double no of chr) (4N) → replicate DNA so contain chromosome diploid no (46/4N)

1st meiotic division → 2ry spermatocytes (diploid no of chr.) → 2nd meiotic → Spermatids (haploid no) — 23 chr

4N, 2N, 1N

23 كل واحد فيهم → عند التخصيب → Zygote (46)

* **Spermiogenesis phase** → عبارة عن Maturation changes occur in spermatids → Mature sperm

These changes include:
(3)
- Formation of Acrosome
- tail
- Nuclear & cytoplasmic changes

→ Spermiogenesis

- **Formation of Acrosomal** the acrosome is a modified bag: contains Lysosomal Enzymes that are necessary for penetration of ovum, Golgi apparatus, gives the material for **acrosome formation**.
- The formation of this acrosome passes by (6) stages (Sa, Sb1, Sb2, Sc, Sd1, Sd2) to form the **acrosomal** cap at the anterior pole of the sperm. pushing the nuclear peripherally.

* **Formation of The tail** → "تشكل الذيل"

→ The Centricles migrate to The other Pole (peripherally). To form the tail, at the same time the mitochondria migrate to the same Pole & arranged spirally around The tail to supply the The sperm (E)) energy (source of energy).

* **Nuclear & Cytoplasmic changes** → تغيرات

as aresult of formation of the acrosome the acrosom. at Cap pushes the nucl. peripherally so it come Flattered (E) Condensed chromatine.

The Cytoplasm is ↓↓ (to) form the "residual body" Thatis Phagecytosed by the Sertoli Cells to complete The process of formation.

Lang ① Nucl: flattened peripheral condensed chromatin
② Cytopl: ↓↓ → residual body

clinically important points

Time needed for complete Spermatogenesis (from (AP)) tail formation of mature sperms is about (74 ds)

+ 16 ds needed for maturation in epididymis

= 90 ds (so time needed for any lit of in full course = 90d

NB

- No of sperms produced daily

  70 million

Sertoli

- Nucleus → Chic Tri partite
- BM < laminin, Type IV Coll — formed by < Sertoli Cells & Myoid Cells
- Cytoplasm . extensive Golgi app. . SER & lipid droplets (Correlating e⁻ steroid synth).
  . with r ER.
- Cytoskeleton :
  - Actin filament
  - Intermediate " (Mainly Vimentin) → (protect against Mechanical forces)
  - Microtubules → . directionalized Transport in Cells.
    . assist to Maintain Architecture
    . entrenchment & then release of Spermatids

(Source: Spermatogonia)

Tubulobulbar Complexes & Ectoplasmic Specialization

• Control (Regulation) of Testicular Function (Nieschlag 2010)

- **Endocrine = Central or Hormonal Control**
  - (By) Hypothalamic-pituitary axis or Hormones → play central role in regulation of testicular Function.
    - GnRH
    - FSH
    - LH
    - Prolactin
- **Paracrine / Autocrine = local regulation or Control**
  - important for (modulation) of Endocrine (Hormonal) Activities by certain locally produced Factors
    - Steroid Hormones
    - INSL-3
    - Growth Factors
    - Immune System Factors.

Central Endocrinal Control (HL) "Nieschlag"

- GnRH
  - Structure
  - Secretion
  - Mechanism of Action
- Gonadotropins:
  - Structure
  - Secretion
  - Mechanism of Action.
  - Role of FSH & LH in Spermatogenesis.

VIAGRA
sildenafil

Control of

* Function of Testis (2) — spermatogenesis / steroidogenesis
is controlled by 2 mechanisms — pretesticular control / Testicular control

* Pretesticular control: by Hypothalamic – Pituitary – Testicular interaction

Hypothalamus: sec. → GnRH = LHRH (gonadotrophin releasing H)
(from) arcuate nucleus ... secreted at pulsatile manner every (60–90 min)

↓

Pituit. ++ Release of LH & FSH from Pituitary (pituitary gonadotrophins)

(FSH) ↓ action → Sertoli cells
↓ ++ Sec. of:
* Androgen binding Protein ↓ Bind to Andr. secreted from Lydig cells → High conc. of And.
* inhibin ↓ inhibit FSH release from pituit.

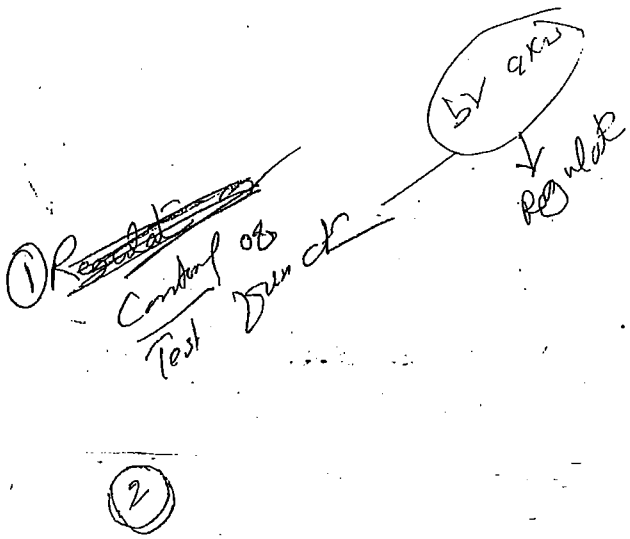
(LH) act on → Lydig cells ↓ Androgen ↓ Germ cell maturat.

N.B: FSH & LH acting by activat. of "adenyl cyclase syst."

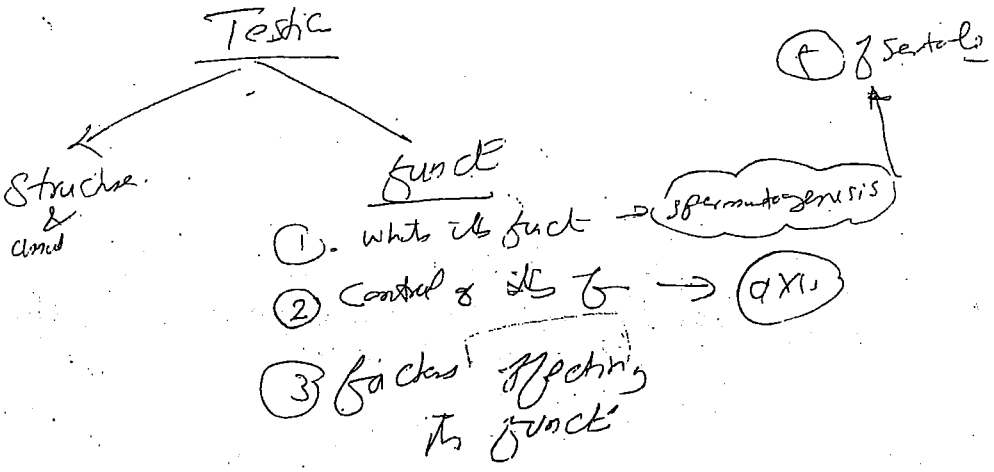
Role of PRH ??

(•) Prolactin: PR
→ physiological NL level → ↑ conc. of cholesterol esters.
→ pathological high level → Impaired Testicular functn either by:
- ↓ pituit. response to GnRH
- or ↓ 5-α reductase activity

(+) Potentiate eff. of LH on Lydig


(1) Regulate
Control of
Test
by axis
Regulate

(2)


Testis
Structure & canal
Funct
(1). what is funct
(2) Control & its
(3) factors affecting its funct
spermatogenesis
(4) Sertoli
axis

Testis → Androgen ... (Gonadal Hs) ...
... Regulation of this axis or control

+ve (stimulatory) feed-back

- Hypoth. Hs → + Pituit Hs → ++ Gonadal Hs

- ve (inhibitory) feed back ..

(a) Aromatization of Gonadal Androgen to Oestrogen:
→ (−) GnRH release
→ (−−) LH

(↓LH) GnRH

(b) Inhibin → − − FSH release.

Factors that aff. the testicular function .. :
1. Age
2. Temp
3. Vasculature

1 # Aging aff ← sexual response cycle, Testicular function ..

↓ effect is: «on»

Germ cell S.NT

at 40 y.o → 85% of Semi-nef. tubules show NL spermatogenesis. —

after 70 y.o → 30%
after 80 y.o → 15%
.. of tubules show
↓ Volume ..
↑ thickness of wall
↓ sperm product

Sertoli

at young adult, testis contain 500 million Sertoli cells
↓
after 50 y.o, ↓ by 50%

Leydig ..

after 50 y.o.
1. ↓ No by 50%
↓
« Testosterone »
2. ↑ SHBG
↓
↓ Free testosterone
3. ↑ peripheral conversion of And. to Oest.
↓
↓ A/O Ratio

↓ NO ↑ SHBG ↑ Conv. (O/A) ↓ A/O

BPH.
Gynaecomastia } in old.

2 Temp. Testicular Temp is 34 °C
< 3-4 °C below Body Temp
< 1.5-2.5 °C below Scrotal Temp.

Reg. of Temp By .. (2 factors)
34 °C
2 < 2 mech.

4

VIAGRA sildenafil

(A) Scrotal skin that — Thin .. No S.C. f— / Rich in ~~blood supply of~~ sweat gland / Large surface area that: / Controlled by dartos (relax. & cont.)

(B) Counter current heat exchange: depend on specialized arrangement of pampiniform plexus of veins .... that cools the blood before reaching the testis ... (by surrounding coiled veins..) *

low temp... is essential ?? because

A B 2 cells (1) Spermatocytes & spermatids are heat sensitive & degenerate at low temp.

2 pt (2) Some ptns as Androgen binding ptn. & Ptn G (that is essential for (FSH function...) Are active only at 34 °C. — A C epididymis

2 ⊙ 2 pathological conditions are Ass. ē disturbance of Testicular funct. i.e. disturbance in Temp are

⊙ undescended testis

⊙ Varicocele ....

3 Vasculature (microcirculation) — despite of Total testicular blood flow is constant ... There is a great variation in the flow among the different regions of testis ... & According to the dilatation & constriction of Terminal arterioles...

H A g

* Exact mechanism by ū testicular micro-Circ. may aff. the testicular functions is Not yet established

* many studies state that the Circ. may play a role in infertility & possible Role of Chronic Testicular ischemia in

Local Regulation of Testicular Function

(paracrine / autocrine regulation)

- There are (3) Types of local interactions & communications bet. different testicular cells which are mediated by "Certain Secretory Factors":

1. Paracrine: Factors acting between Neighboring cells (by diffusion)
2. Autocrine: factors which are released from the cell & work back on the same cell.
3. Intracrine: Factors never leave the cell & its site of production & action is the same.

- Sertoli cells were viewed as coordinators & regulators of Germ cell development & maturation; Recently they are now believed to be influenced by Germ cell products that can influence the secretory activity of Sertoli cells.

So: Sertoli cells are under the local control of germ cells

- These Paracrine / Autocrine / Intracrine factors mediate the communication between:
  - ✓ Interstitial & tubular compartments
  - ✓ Sertoli & Germ cells.
  - ✓ Germ cells & " ".
- these factors include:
  1. Steroid Hormones
  2. INSL-3
  3. Growth Factors
  4. Immune factors.

1. **Steriod Hormones:** T is the main secretory product of Testis.

T.

(Intratesticular Conc. is 80 fold > Serum T.)

it acts through 3 ways

- **as T.** → affect Spermatogenesis (mainly Needed For initiation or Spermatogoniogenesis).
- **as DHT** (under effect of Testicular 5-α reductase) → No detected role in spermatogenesis (So Dutasteride no effect on semen)
- **as Estradiol** (under effect of Testicular Aromatase) → it's deficiency has no role in spermatogenesis.

كل مراحل Spermatogenesis

T. Receptors found in:
- Sertoli Cells
- peritubular Myoid Cells

Leydig cells (T) ب Sertoli Cells علاقة

1. Sertoli Cells: Secrete ABP ← binds T. in the interstitium → prevent its rapid metabolism to E. & transport it inside SNT to maintain High Conc. Needed for Spermatogenesis.

FSH → ABP → ↑ Tubules, ↑ Cadherins, FSH ++ T

2. FSH reinforce T. Action (FSH recombinant → ↑ T. production). transport of germ cells

3. T. → ++ Cadherins production by Sertoli Cells (so if ↓ T → affect spermatogenesis)

FSH++ ⇄ ++T

2. **Insulin-like Factor-3** — Regulation

- relaxin-like proteohormone produced by Leydig Cells
- May play a role in regulating Spermatogenesis.

3. **Growth Factors:** e.g by Sertoli cells:

(transforming growth factor)

- IGF-α & β (α → ++ Testis / β → --)
- Inhibin → ++ Spermatogenesis (Recently Serum level Correlate ↑ Spermatogenesis & Testicular size) → So used as Endocrine indicator of local spermatogenic defect.
- Activin → ++ "
- IGF-I, EGF → ++ Spermatogenesis.

4. **Immune System Factors:**

- Some Cytokines produced by: WBCs, Macrophages & Mast cells → affect Spermatogenesis e.g
  - TNF
  - Leukemia Inhibiting Factor (LIF) → role in Sertoli-Germ interaction
  - IL-1
  - SCF (Stem cell Factor, produced by Sertoli)
  - MIF (Migration Inhibition Factor produced specifically by Leydig cells).

(Spermatogonial differentiation & development.)

TNF
IL-1
LIF
MIF
SCF

Epididymis

(Epi = above / didymos = Testis)

Anatomy (4cm L / tubules = 4m)

- Coma shapped structure that overlies the Sup & post lat. Aspects of testis.

Formed of 3 segments:

(1) Head (Caput) : at upper pole of testis.
(2) Body (Corpus) : at post aspect of testis.
(3) tail (Cauda) : attached to inferior pole

- **Length** < 4 cm uncoiled tubule : 6/meter

- **Structure**

(1) **Head**: . formed of 12-20 tubules (Efferent ductules) that arise from Rete testis

those tubules are very convoluted to form conical masses called lobules of epididymis. 20 together forms the head.

Each lobule Composed of single Convoluted duct ≃ 20 cm Long.

duct of epid. encapsulated by fibrous sheath That sends septa → divide the duct into Histologically similar areas.

(2) **Body & tail**: Efferent ductules → become one duct called (Epididymal duct)

This duct very convoluted at body & tail end

Called Epid. tail → Vas deferens.

- Blood Supply : testicular, Vasal & Cremasteric artery
- Venous drainage : to papiniform plexus of Veins.
- Nerve Supply : Sympathetic
- Lymphatic drainage: Head & Body (as testis) → Para-aortic L.N.
  tail (as VD) → External Iliac L.N.

Function of Epididymis:

4 Main functions

1. Sperm Storage & Concentration
2. Sperm Transport
3. Sperm protection
4. Sperm Maturation

- eja culated sperms [illegible] epid. & then

- Sperm Concentration ability of epid. (i.e.) due to fluid reabs subsequent to antiluminal Electrolyte transport...

- Epididymis act as a duct that transport the ~~Testes~~ sperms via:

(1) Hydrostatic pressure from fluid secretion in testis. (by Sertoli)

(2) Motile Cilia of epididymis.

(3) Rythmic Contraction of Myoepithelial Cells.

(A) Sperm ← Conc. / Storage / Transport

(B) Protection.

(C) Maturation.

[3] Sperm protect — through secret of:

Anti-proteases (A). protease inhibitors: (-- proteolytic degradat-)

Antioxidant (B) Anti-oxidants (Glutathione peroxidase)

Become (C) Blood Epididymal barrier (Helped by Cadherins)

Anti- (D). Suppressive factors (↓ Lymphocyte &/ Complement activat-).

↳ immunosuppressive

[4] Sperm Maturat- (W)

Means 2:

Attainment of Motility [Sperms of tail have More Velocity > that of Head of epid.]

Some epidid. products are secreted have role in Sperm Motility:

(1) L. Carnitine: protein that's not synthesized by epid. but Concentrated From circulat- inside epididymis.

(2) α-glucosidase: enz
- secreted from Epid.
- a Marker for epididymal
- (neg) of Epididymal Obst.

Attainment of Fertilizing Capicity

(Fertilizing ability of Sperms & tail > Sperms of Head & bod)

through sec. of:

(1) Zinc

(2) Sialic acid: essential For integrity of

ess. For Matural (3). Inisitol

(4) Glycerop Phospho Choline

(5) D. Galactosidase (++ Spermatozoum binding)

(6) appearance of Maturational Antigens:

. Maturational Ags. are surface structure found only on Epid. as

① P34H protein (sperm ovum interact protein . IVF succ depends on sperm content of it).

MMP ② Major Maturational protein (MP52)

③ Sperm integral Memb. protein (PH 20 30)

Control of Epididymal Function

(1) Hormonal : ABP & SHBG can regulate the funct by conversion to DHT.

(2) Neural : Sympathetic Mediated (Sympathetic denervat → ↓ sperm Motility)

(3) Thermal: High temp. as in Varicocele / Cryptorchidism → Epid. dysfunct.

What are the Epididymal Markers:

(Not sure d.t variat in level even in fertile Men)

- α-glucosidase
- L-carnitine
- Glycerol phosphocholine.

Male Accessory Sex organs

- ~~Epididymis~~
- VD
- SV
- prostate
- Glands

((VD))

** . def:- 2 tubes or ducts Cnnecting the R.t & L.t epididymis to the ejac. ducts

** . Length : 35 cm

** . start at : epididymal tail ⟶ enter the Inguinal Canal in the post. part of spermatic Cord ⟶ Int. ring ⟶ Pelvis < over the Ureter / post. to " UB (med. to SV).

** . ends by enlargment & dilatation ⟶ Ampulla of Vas ⟶ join ducts of SV ⟶ ejaculatory duct formation

** . Clinically it divided in to 5 points:

1. Epididymal part : inside Tunica Vaginalis.
2. Scrotal " : outside Tunica (Site Vasectomy)
3. Inguinal
4. Pelvic part
5. Ampulla of Vas.

. Blood supply : Vasal a. (br. from Inf. vesical a. & br. from Int. iliac).

. Venous drainage: Vasal veins.

- Function: during ejaculation → (Reflex. Contraction of Smooth muscles → peristalsis → Expell of Spermatozoa to Urethra.

- Clinical importance → Male Contraception

① Vasectomy (irreversible)

② Vasoocclusive Contraception (Reversible):

Vasal ⟨ clips / plugs / Valves → (Silicon plug block the Vas)

③ Reversible inhibition of sperm under Guidance: injection of gel material that Coat the Vas from inside → disturb the sperm during passage through the Vas by unknown mech. ±

(RISUG)

① change of pH → Kill sperms

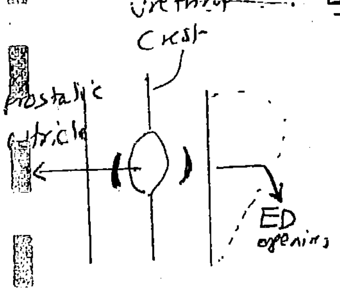
② rupture of sperm memb. & disturbed sperm charges

③ disturbe the -ve sperm discharge → prevent Zonal binding.

SV

Anatomy: 5-10 cm, 2 lobulated glands at post. lat aspect of UB (& lat. to Vas)

- Not felt by PR except if ⟨ inflammed or Obst.
- each gland has a duct & Join Ampulla of Vas → ejaculatory duct (ED)
- ED = 2 cm, open on post wall of prostatic urethra by slit-like opening on the summit of Urethral crest called (Verumontanum = Colliculus Seminalis)



post. wall of Prostatic urethra

Funct^ كتير مهم

1. **Semen Vol**: its secret^ represent 60-80% of semen vol; 2nd fract^ of split ejaculate.
2. **Alkalinity** of Semen
3. **Coagulat^ & Viscosity** of Semen
   - Coagulat^ d.t protein kinase = Seminogelin I
   - Viscosity d.t SV proteins (NL semen is viscous)

So SV Hypofunct^ ⟨ ↓ Coagul^: ↓ mot. ; ↑ Viscosity: ↓ mot & ↑ chromatin stability

NB: Semen ejac. → liquid → coagul^ → liquefact^ (مهم) → allow contact of sperms ē ingredient of semen e.g ↑ motility, ↑ chromatin stability & # Autoimmunity in ♀ tract.

4. **Fructose secretion**:
   - Androgen dependent
   - Main source of energy for sperms.
   - NL level: 120-450 mg
   - Abnlly ⟨ ↑↑ in: Azoo or oligo; ↓↓ in: NL, Poly Zoo, SV. obst or Absence, EDO.
5. **Other Secretory F**: (i) NO (↑ AR ↓ ↑ fructose) (ii) Protectin: VitC, Vit A, SOD ↓ BY ↑ copper e.g smoking. (iii) Zinc (it ↓↓ of chromatin → allow decon → NL fertil, ...)

Prostate

. **Anatomy**: . Cone shapped pelvic structure that encircle the UB Neck & Urethra

. has — Base: at Bladder Neck
— Neck: at Superficial facia of urogenital diaphragm.

... (Funct) كتير جداً

[A] Semen Vol. represent 15–30% of Semin Vol; 1st Fract of split ejaculati

[B]. Acidity of Semen (PH 6.5) — مسؤول عن Liquifaction < 30 min

[C] secretory products:

[1] Prostate Specific Proteins = (Proteases = fibrinolysins)

- PSA (Seminin)
- PAP (prostate acid phosphatase)
- PBP (prostate binding Protein).

↑ in Cancer prostate, PR; Prostat. — تعمل Liquefact.

أهم حاجة . Action → proteolysis (major function of prostate Sec.) (Semen Liquefact.)

شفهي — ماهي Markers of:

1. Epididymis
2. SV
3. prostate
4. Testis

ماهي وظيفة ال

- Zinc
- Fructose.

[2.] Citric acid

. maintain osmotic equilib. of sperm
. Motility
. it ± Ca+ → prevent Calculi

[3.] Spermin: protein

[. Musk odour — Ch. odour
[. Antibacteria

NB ← [4.] Zinc:

. the main "prostatic Antibact. factor" (PAF)
. Sperm motility
. Spermatogenesis
. NL Test. [illegible]
. ↑ Chromatin Condensation

(1) Conc. in Semen = 10 Conc. in Blood
(2) Highest Conc. at sperm tail
(3) Role in Chromatin decondensat.

Glands.

- Cowpers (Bulbo urethral) glands
- Littre's (periurethral) "

def → one of Exo Crine glands, present in the reproductive system of Human male (Homologous To ♀ Bartholin gland).

Site: Pair of pea-sized glands on (Post. lat) aspects of membranous urethra (at base of penis) bet. 2 Layers of fascia of urogenital diaphragm & enclosed by fibers. of Ext. urethral Sphincter. Their ducts open into Bulbous urethra.

- duct: 2.5 cm. (excitation وقت الإثارة)
- Sec → Pre-ejaculate or pro semen ("سائل" slightly) "during sexual Excitat°."
- def: Clear, Colourless, viscous Fluid emits from urethra of males during Sexual excitation.
- Source: [mainly: Cowpers ± : Littre's
- Amount: Variable (0-5 ml).
- Composit°: ① Contain some Chemicals of Semen as acid phosphatase. → ++ Liquefact°

② Sperms → Controversy → but ± Content semen of sperms from previous ejaculation that remains

④ Plasminogen-activator → "Role in" Coagulat° (-- Coag)

Function

1. Lubricate < urethra : for passage of sperms.
   penis : during sexual intercourse.

(لاحظ) 2. Neutrilize acidity of urine & vagina.

3. Flush out any residual urine or FB
4. Pick up sperms remaining in urethral bulb from the previous recent ejaculate. (So ± → pregnancy).

5. Role in semen coagulation. (Anti coagulating effect of plasminogen activator).

Medical problems related to it

1. may → pregnancy (controversy).
2. Transmit HIV
3. overproduction may be a complaint of many men, (D.D. prostatorrhoea in (??) sec. ass ē defecation & urination & not related to sexual excitation as in prosemen).

(انتبه)

NL amount : 2.5 ml

لغوريس ∂ prosemen & prostatorrhea

4. Religious attitudes : → purification التطهر

(لازم نشوفها) (لانه مين بيغسل)

(Rx) of overproduction :
Some reports → (Rx) by Finasteride

Littre's glands : present & open throughout penile urethra . secrete : mucus

Function of prosemen

Physiology of sperm.

Structure: → human sperm is ≃ 60 μm in Length (60 μm) & in Contrast to other body Cells it is flagellated & has (no) Cytoplasm

تقسيم ٣ اجزاء
- Head (4.5 μm) — Head 4.5, 3 μm
- Neck (0.5 μm) (Conn) 0.5 (40–70%)
- tail (55 μm) ← 55 μm

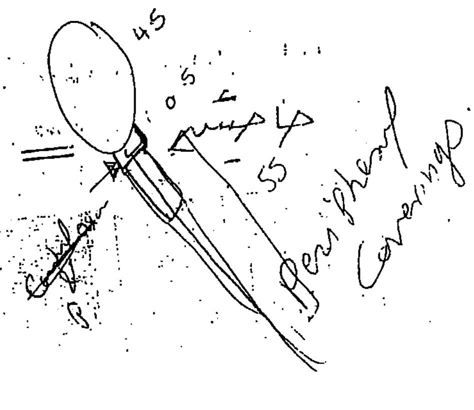
Head, oval in shape (4.5) μ in Length & 3 μm in diameter, Contains the nucleus that is surrounded anteriorly by the acrosome → ساتريف

↓ modified bag or membrane bound organell that is formed by Golgi apparatus, Contains Lyzosomal enzs w necessary for ... it occupies the ant (50–70%) of the sperm head infront of the nucleus.

Neck (Connecting piece) — عشان توصل Tail & Head — ... it is the post extension of sperm head that connects the head to the tail (flagellum).

Tail formed of Central Core (Called axonem) & peripheral Coverings (w) are different from each piece of tail.

Axoneme (Central Core) =



Axoneme by EM

2 Central Single Microtubules + 9 peripheral doublet Microtub.

Surrounded by protein ring ω is attached to the peripheral Microtub. in a radial manner (radial spokes, links) like the wheel

each of 2 Microt. of the 9 termed A & B

each A Contains (2) arms (dynein arms) the outer one is large

the tail is divided In to 3 Pieces or areas.

Mid Piece → 5 µm.
Main " → 45 µm.
End " → 5 µm.

a) The mid piece: its the area following the neck, the axoneme, in this area is surr. by spirally arranged Mitoch. that act as energy source for the sperm. (5 µm)

b) The Main or principle piece: (45 - 50 µm) the axoneme here is surr. by fibrous sheath, its devoid of Mitochond.

c) the End Piece (5 µ) this the distal end, the axoneme is not surrounded by either Mitochondria or fibrous sheath.

1. Mid piece → surr. Mitoch. ✓
2. Main → Fibrous sheath ✓
3. End → no Mitoch. or fib sheath ✓